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SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

Seventy-seventh meeting.

Cornell University Medical College.
President Jacques Loeb in the chair.

1 (1179)

A note concerning strains of Treponema pallidum obtained from the brains of paretics at autopsy.

By **J. A. F. PFEIFFER** (by invitation).

[*Government Hospital for the Insane, Washington, D. C.*]

For the purpose of obtaining information which might assist in elucidating some of the problems regarding syphilis of the nervous system, and determining whether there is any possible foundation for the assumption that a neurotropic strain of the *Treponema pallidum* exists, an endeavor has been made in this laboratory to secure strains of the *Treponema* from the brains at autopsy of cases with parenchymatous syphilis (paresis), by inoculating rabbits with the cortical material; and we have been successful in producing typical lesions in the testicles of rabbits, in which treponemata could be demonstrated.

It would seem pertinent to review briefly the observations of Noguchi in his work on rabbit syphilis. He distinguishes a difference in the morphology of several strains of *Treponema pallidum.* Some of the strains appear notably thinner than others, and this variation in morphology seems to have some distinct relationship to the degree of motility, the infectiousness and facility of cultivation. Three different forms are described. A thick form having a width of 0.3 of a micromillimeter, a somewhat thinner one with a thickness of 0.25 of a micromillimeter, and a thin form 0.2 of a micromillimeter in breadth.

According to Noguchi hard, indurated and sharply defined

nodules are produced in the testicle by the thicker forms in five to six weeks. With the thinner types, however, the incubation period is briefer and in ten to fourteen days the testicle becomes swollen, gradually resulting in a large diffuse lesion.

In reference to the investigations which have been made with strains from the nervous system, it is interesting to note that recently Zinsser has obtained contradictory results to those of Nichols with the identical strain sent him by Nichols. Wile's work with cultures of his strain seem to support the results of Zinsser.

Rabbits were inoculated with material from the brains after death of seven cases of parenchymatous syphilis, and we have succeeded in obtaining strains in four cases.

Two of these strains were lost after the second generation, but of the two remaining, we have been able to continue one to the seventh and the other to the ninth generation. Although these strains have not passed through so many generations, lesions have occurred in a sufficient number of rabbits of each generation to afford some interesting deductions. In reference to the percentage of takes, there has been a slight variation in the two strains studied. While one of the strains has produced lesions in eighty to ninety per cent. of the rabbits inoculated, the other has shown a fluctuation between seventy and one hundred per cent. The incubation periods have likewise exhibited some irregularity. So far they have varied from sixteen to sixty-seven days, but have averaged from nineteen to thirty-one days. Neither of the strains have shown any distinctive features in regards to the incubation time. The character of the lesions has constituted a factor of considerable significance, in that both hard nodules and large diffuse processes have been obtained.

With one of the strains, a majority of the lesions which developed were of the large diffuse variety. It is apparent that in this instance most of the lesions have corresponded to those produced by the so-called thinner forms of treponemata.

The inference seems justifiable, from the work of our laboratory extending over a considerable period of time, that the strains of treponemata designated by Noguchi as the thinner types appear to play as important a rôle in parenchymatous syphilis of the nervous system as the thicker ones.

There would appear likewise no reason to assume that a so-called neurotropic strain exists, but that in syphilis of the nervous system the different forms of the *Treponema pallidum* may be encountered.

2 (1180)

A sex-intergrade strain of Cladocera.

By ARTHUR M. BANTA.

[*From the Station for Experimental Evolution, Cold Spring Harbor, Long Island, N. Y.*]

The above-mentioned strain appeared about a year ago. For four years the writer had been breeding a number of strains of *Simocephalus vetulus*. For 130 generations reproduction was entirely parthenogenetic. The young were all females, there being neither males nor sexual eggs. In the 131st generation of one of the eleven strains of the species reared under laboratory conditions for so long there suddenly appeared, in addition to normal females, males and *sex intergrades* of many sorts. This strain has continued to produce sex intergrades for a year—more than twenty generations—and the character of the intergrades produced does not seem different now from what it was when the sex intergrades first appeared.

In this species, in addition to the character of the gonads, eight morphological secondary sex characters are recognized. In the sex-intergrade strain the sex array may be roughly classified into normal females, female intergrades, hermaphrodites with various combinations of male and female secondary sex characters, male intergrades, and normal males.

The female intergrades range from females with a single, perhaps poorly developed, male secondary character to those with all the secondary sex characters male. The hermaphrodites have various combinations of male and female secondary characters. There are male intergrades with as many as five secondary sex characters, though ordinarily the male intergrades have only one or two female characters.

The male intergrades usually have incompletely developed reproductive organs. Sperm is produced in various amounts.

The amount is usually smallest in the male intergrade with the larger number of female characters; and usually males with a single female secondary sex character produce fewer sperm than normal males. The female intergrades with one or two male secondary sex characters usually possess a high fertility, those with as many as four or five male characters are in general much less prolific, while those with six or more male characters are usually sterile or nearly so. The hermaphrodites are frequently sterile, though some are moderately prolific. The normal females within the sex-intergrade strain produce representatives of the entire sex array. In general however the female intergrades with several male secondary characters produce a higher percentage of males and male intergrades than either the normal females or the female intergrades with few male characters.

Sex here appears as a purely relative thing. There occurs practically every gradation from the entirely normal female with a full complement of female secondary sex characters; through female intergrades of all sorts; hermaphrodites, with various combinations of secondary sex characters; and male intergrades of various rank; to normal males with all the primary and secondary sex characters distinctly and strongly male.

3 (1181)

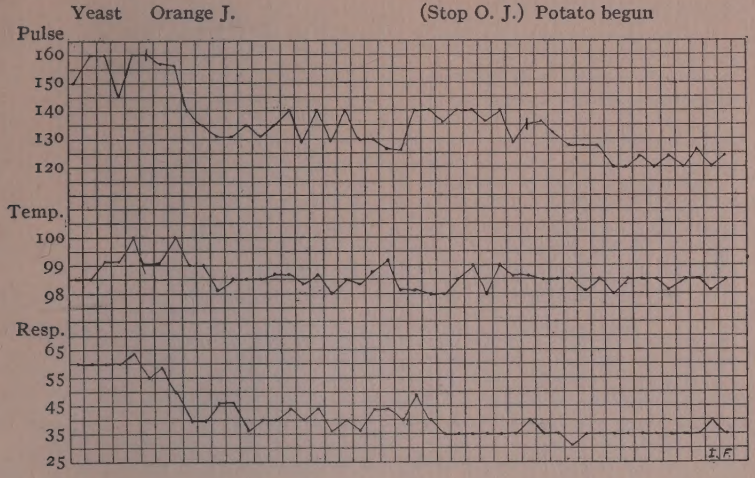
Cardio-respiratory involvement in infantile scurvy.

By ALFRED F. HESS, M.D.

[*From the Board of Health Laboratories, New York City.*]

Infantile scurvy is commonly regarded as a disorder which affects the blood vessels and the bones. In previous communications it has been shown that this view is too narrow, that the heart is frequently enlarged, the deep reflexes exaggerated, and that there may be changes in optic discs. In the present communication we wish to point out that even in moderate instances of infantile scurvy, there may be found marked polypnea and tachycardia. The accompanying chart illustrates this condition and demonstrates likewise its scorbutic nature by the promptness with which it reacts to antiscorbutic diet, to orange juice or to potato.

This pathological syndrome evidently is due to an involvement of the pneumogastric. It is interesting as another proof of the important part which the nervous system plays in this disease,



and in associating it still more closely with other so-called "deficiency diseases" such as beriberi and pellagra, in which the disturbances of the nervous system seem to be primary in nature.

4 (1182)

On the question of the transformation of fibrin into fibrous tissue in tissue culture preparations.

By R. A. LAMBERT, M.D.

[*From the Pathological Laboratory of the Presbyterian Hospital, New York.*]

During the past year there appeared a paper by Baitsell[1] working in Professor Harrison's laboratory at Yale University describing changes in the fibrin clot of tissue culture preparations, which he interpreted as a transformation of the fibrin meshwork into fibrous tissue. Extending his observation to the living animal this author has described[2] in the healing wounds of frogs similar changes in the fibrin which early fills the wound and concludes that instead of forming a temporary scaffolding to be removed

[1] Baitsell, *Jour. Exper. Med.*, 1915, XXI, 455.
[2] Baitsell, *Jour. Exper. Med.*, 1916, XXIII, 739.

later, the fibrin becomes transformed into permanent collagen fibrils such as are found in the healed scar. This view is so at variance with that generally held that a careful review of the work seems desirable.

The changes in the fibrin referred to may be briefly described. When the tissue culture is first prepared the fibrin meshwork of the clot is so delicate that the coagulum appears as a homogeneous almost translucent mass. Within two to five days as the clot contracts there appear in a certain number of the preparations, coarse fibrils sometimes wavy in character which radiate generally from the central fragment of tissue. We have observed this change in clotted fowl, human and rabbit plasma, as well as in frog plasma studied by Baitsell. The formation of these coarse fibrils is evidently the result of the contraction of the clot with fusion of many of the delicate fibrin threads. The change may be facilitated, as Baitsell has pointed out, by mechanical disturbances such as loosening of the clot at certain points. In human pathological material one sees a similar formation of coarse fibrils wherever fibrin in any quantity is deposited as for example, in fibrinous pleurisy, peritonitis, thrombi, pneumonic exudate, etc.

Baitsell's interpretation of this change in the fibrin clot as a transformation of fibrin into true fibrous tissue was based on the physical character of the unstained fibres and their reaction to connective tissue stains. Chemical tests were also applied. In their physical character the newly formed fibrils resemble collagen fibrils, but in the opinion of the author and of those to whom the preparations were shown the resemblance is superficial and is certainly not important in a differential study of this kind.

The stains used by Baitsell were those commonly employed to differentiate connective tissue: Van Gieson's picro-fuchsin, which stains connective tissue red and fibrin yellow, and Mallory's fuchsin-anilin blue with which connective tissue is stained blue and fibrin red. Baitsell obtained negative results with Van Gieson's stain, but with Mallory's stain as modified by Mall[1] the coarse fibrils appeared an ultramarine blue in contrast to a purplish blue of the fine fibrin threads.[2] In my hands this modified stain

[1] Mall, *Amer. Jour. Anat.*, 1901, I, 329.

[2] Baitsell states that both the modified and unmodified Mallory stain was used but his descriptions appear to apply only to the results obtained with the modified stain.

has not proved to be a differential stain at all since even the freshest fibrin takes a bluish hue. The difference between the coarser and finer fibrils appears to be one of intensity of stain. On the other hand the original Mallory stain as well as the modification made by Mallory himself in 1905[1] differentiates sharply between fibrin and connective tissue. The coarse fibrils as well as the fine fibrin strand taking a rich orange red in contrast to the deep blue of the connective tissue fibrils. I have used still a third connective tissue stain, the Bielschowsky silver method, which is regarded as a more delicate stain even than Mallory's. This method gives the same results as the others, that is, the fibrils react as fibrin and not as fibrous tissue (fibrin, dirty brown; connective tissue fibers, deep black).

My results with chemical tests—digestion with weak acid and pancreatin—agree with those of Baitsell. The coarse fibrils under question are readily dissolved, indicating their fibrinous character.

It has therefore been concluded that the only support for Baitsell's transformation idea consists of results obtained with a modified stain which does not differentiate fibrin and fibrous tissue. Chemical tests and reactions with all three of the differential connective tissue stains in general use show that no such transformation takes place.

5 (1183)

A comparative study of different methods of performing the Wassermann test for syphilis.

By J. WHEELER SMITH, JR., and W. J. MACNEAL.

[*From the Laboratories of the New York Post-Graduate Medical School and Hospital.*]

Wassermann tests were performed by three methods upon 496 identical specimens from 477 patients. In the first method a cholesterin-reinforced antigen was employed and the first incubation was carried out at 37° C. In the second method a simple alcoholic extract was used as antigen, with incubation also at 37° C. In the third method this latter antigen was again employed, but the first incubation was carried out in the refrigerator for a period of four to twenty-four hours.

[1] Mallory, *Jour. Med. Research*, 1905, XIII, 113.

The last method proved more sensitive in the group of known syphilitics than the other procedures tested. Furthermore, a positive result thus obtained proved to be more trustworthy evidence of syphilis than did positive results obtained with the cholesterinized antigen and first incubation at 37° C.

6 (1184)

The influence of alkali upon the glycosuria, hyperglycemia and carbon dioxide combining power in human diabetes.

By **J. R. MURLIN** and **L. F. CRAVER** with the Clinical Co-operation of **W. L. NILES** and **WARREN COLEMAN.**

[*From the Physiological Laboratory of Cornell University Medical College and the Second Medical Division of Bellevue Hospital, New York.*]

Murlin and Kramer[1] have shown that alkali administered to depancreatized dogs reduces the glycosuria, often lowers the blood sugar, and, especially in partially depancreatized animals, assists in the combustion of glucose. It has been held generally that alkali administered to diabetic patients does not influence the glycosuria[2] or hyperglycemia. A critical study of several cases kept under perfect dietary control in the metabolism ward of the Sage Institute of Pathology in Bellevue Hospital during the past summer seems to show, however, that alkali (1 per cent. Na_2CO_3) administered by duodenal tube often reduces the glycosuria very materially and may likewise affect the hyperglycemia.

A preliminary study of the blood sugar and carbon dioxide combining power of the whole blood in six patients with diabetes and several normal persons exhibits a striking inverse relationship which is almost proportional.

Two patients among the eight studied exhibited features of special interest. One (Frank B.) had a normal blood sugar throughout but excreted from 25 to 39 gm. of sugar, regardless of the amount eaten. He is probably a case of renal glycosuria. The

[1] Murlin and Kramer, *Journal of Biological Chemistry*, 1916, *Proceedings of American Society of Biological Chemists*, XXIV, March No., p. i; Full report, *Ibid.*, 1916, XXVII, Nov. No.

[2] Von Noorden, Handbuch der Pathologie des Stoffwechsels, II, Berlin, 1907, p. 576.

other (Harry H.) was sugar-free for two weeks on 15, 30 and 50 gm. carbohydrate but had no pancreatic digestion. When given pancreatic digestion with lactopeptine[1] (New York Pharmacal Ass'n) or Merck's pancreatin he excreted about 60 out of 100 gm. carbohydrate fed. Proof that such digestive powders do survive the stomach was established by recovery of them from the intestine by means of the duodenal tube. The utilization of carbohydrate under these circumstances was apparently increased by giving 0.3 per cent. sodium carbonate by duodenal tube.

7 (1185)

The influence of radium emanation on the activity of vitamine.

By CASIMIR FUNK.

[*From the Memorial Hospital, New York.*]

The present investigation was undertaken with the view of finding out a method of differentiation between the vitamine which cures beriberi and the vitamine which stimulates growth of young rats. It was also of interest to ascertain whether an unstable substance of the vitamine class would undergo inactivation under the action of emanation. This latter point is of practical importance in view of the extensive use of radium in the treatment of cancer. In the light of modern knowledge of the subject the therapeutic use of radium for the above purpose can be explained by a stimulation of leucocytes or by a destructive action of a physiologically active chemical substance, the second view not being supported by actual experiments; so for instance the alleged action of radium on lecithin with the liberation of choline was not confirmed by modern investigators.

The experiments were performed in the following way. Autolyzed yeast was subjected to the action of radium emanation (the activity of which and the time of action has been determined by Dr. Bosworth from Memorial Hospital, the details of the work to be presented later) samples of the radiated and the non-radiated control autolyzed yeast being injected intramuscularly into pigeons

[1] 10 gm. Lactopeptine produced the same digestion, as measured by the output of sugar, as 5 gm. Merck's pancreatin.

of uniform weight of 300 gr. which developed beriberi on white rice. The dose of the administered solutions was gradually worked down to .1 c.c. which was found to be the minimum dose in both cases. The result therefore was that the radium emanation has no destroying action on beriberi-vitamine.

In a similar way it has been ascertained that radium emanation possesses no action on the vitamine which stimulates growth in young rats so that by the above method the differentiation of the two vitamines has not been accomplished. The method used was the same as that described by Funk and Macallum.[1] Here also the radium emanation was found to have as little action as on the beriberi-vitamine.

Finally the action of radium emanation was tested on Rous's spindle cell chicken sarcoma. An extract of the tumor was prepared under aseptic precautions, which was filtered through filter paper and divided into two portions. In one of the portions emanation tubes with a measured amount of emanation were directly inserted and left for forty-eight hours, the control liquid being kept the same length of time. Both solutions were then injected into the pectoral muscle of a number of small chickens. In both cases tumors have appeared after a delay of 3–5 weeks which shows that radium emanation has hardly any action at all on the agent of the chicken sarcoma even when used in doses exceeding those applied in cancer therapy.

8 (1186)

The mechanism of the diffusion of electrolytes through the membranes of living cells.

By JACQUES LOEB.

[*From the Rockefeller Institute for Medical Research, New York City.*]

When eggs of *Fundulus* are transferred from sea water directly into a solution of a potassium salt a number of embryos will be poisoned during the first hours so that their hearts stop beating. When the eggs are washed for twenty-four hours in H_2O (or any solution of a non-electrolyte) before being put into the same

[1] *J. of Biol. Ch.*, 27, 51, 1916.

solution of potassium salt they have acquired a remarkable immunity against potassium salts. When eggs are put directly from sea water into an *m*/8 KCl solution in one and one-half hours the heart beat stops in two thirds of the eggs; the same effect requires in eggs previously washed for twenty-four hours in H_2O four days, *i. e.*, an *m*/8 KCl solution poisons the embryos of unwashed eggs sixty times as rapidly as the washed eggs.

It can be shown that this difference between the washed and unwashed eggs is due to the fact that the unwashed eggs have some of the salts of the sea water at their surface. If we put washed eggs into *m*/8 KCl solutions made up in H_2O and different concentrations of sea water or NaCl + $CaCl_2$ or NaCl or any other Na salt, the eggs are poisoned the more rapidly the higher the concentration of the sea water or the sodium salt, up to a concentration of about *m*/4. If a slightly higher concentration, *e. g.*, *m*/1, is used, the opposite result is observed; namely, a retardation of the rate of diffusion of KCl into the egg and hence a protection of the eggs. This is the antagonistic salt action which has hitherto exclusively occupied the attention of biologists.

Experiments, which lack of space forbids to enumerate, show that the difference in the rate of poisoning of the embryos mentioned, is due to a difference in the rate of the diffusion of the potassium salts through the membrane. It follows then that for the diffusion of potassium salts through the membrane of the egg of *Fundulus*, *aside from the osmotic pressure of the solution a second factor is required, which we will call the general salt effect, and which consists in a reaction between the electrolyte and a certain constituent of the membrane* (possibly the proteins) *whereby the membrane becomes diffusible for the potassium salt.* If the potassium salt is alone in solution it cannot diffuse into the egg until it has produced the salt effect upon the membrane. This requires considerable time if the concentration of the KCl solution is low and this explains why it takes so much more time for the KCl solution to poison *washed* eggs than eggs transferred directly from sea water into the KCl solution.

A further proof for the correctness of this view is found in the fact that if eggs are poisoned in a potassium salt they cannot recover when put into a solution of a non-electrolyte, while they

will recover in the solution of certain electrolytes. The recovery depends upon the possibility of the diffusion of potassium salts out of the egg and not of the diffusion of the outside solution into the egg, since very toxic solutions of electrolytes, *e. g.*, NH_4NO_3 or $(NH_4)_3$ citrate, may be as efficient in bringing about the recovery of the egg as comparatively harmless or beneficial salts, like NaCl or NaCl + $CaCl_2$ or sea water. The relative efficiency of various salts for the production of the "general salt effect" depends to a large extent on the nature and valency of the anion and is for Cl : SO_4 : citrate = 1 : 4 : 16, *i. e.*, it follows Hardy's valency rule for the precipitation of proteins. This suggests that we may be dealing in this case with an action on some protein. The same valency rule holds not only for the acceleration of the rate of the diffusion of potassium salts but also for the opposite effect; namely, the antagonistic salt action.

Somewhat similar results were obtained for the diffusion of acid into the egg and these experiments seem to indicate that for the diffusion of these two groups of electrolytes, potassium salts and acids, in addition to the osmotic pressure of the substance a second effect is required which we call the general salt effect and which consists in the modification of a certain constituent of the membrane (possibly a protein) by the salt.

9 (1187)

The registration of heart sounds from the exposed heart and large vessels. A demonstration.

By C. J. WIGGERS and A. DEAN, JR.

[*From the Department of Physiology, Cornell University Medical College, New York City.*]

For a number of reasons it was questionable whether the heart sounds recorded from the resonant thoracic wall are composed of the same vibrations as those actually arising within the heart. To assist in answering this question a method of registering the sounds from different spots on the exposed heart and large vessels was devised. The apparatus consists of a sound receptor

stitched to the heart, and a recording Frank capsule. The sound receptor consists of a light segment capsule 2 cm. in diameter and similar to the miniature myocardiograph recently described.[1] It differs in that it has only one arm connected with a trapezoidal plate which pivots on the segment capsule. When stitched to any portion of the heart this arm transmits the sound vibrations to a tensely stretched, heavy rubber diaphragm covering the segment capsule. The interior of the receptor communicates by tubing with a Frank segment capsule covered by a light film of dried rubber cement to which a tiny mirror is allowed to adhere. By leaving a side-tube open to an adequate degree, as is customary in sound registration, the gross mechanical changes are eliminated.

In comparing the sounds thus derived from the ventricle and the aorta essential differences were found, especially in the first sound. The first ventricular sound consists of three elements:

1. One or two initial vibrations which begin during auricular relaxation and precede by a variable interval the rise of intraventricular pressure.

2. The main vibrations composed of 7 to 13 irregular vibrations which begin with the onset of the intraventricular pressure rise.

3. The final vibrations, variable in number, which occur during the ejection period of the heart.

The first aortic sound is also divisible into three components, the second and third of which give the group a configuration essentially different from that of the first ventricular sound. They are:

1. One or two initial vibrations, evidently corresponding to the same vibrations in the ventricular sound.

2. A *first main component* consisting of a group of irregular oscillations beginning at the same time as the main vibrations of the ventricular sound.

3. A *second main component* occasionally consecutive with but often disconnected from the first component so as to give a reduplicated appearance to the sound.

[1] Wiggers, *Amer. Jour. Physiol.*, 1916, XL, 218.

10 (1188)

Histological study of the testes of guinea pigs showing lead blastophthoria. Preliminary report.

By CARL VERNON WELLER, M.S., M.D.

[*From the Department of Pathology, University of Michigan.*]

In an earlier report we have shown that the continued administration of small doses of lead produces a definite blastophthoric effect in male guinea pigs; and that the lead blastophthoria thus induced manifests itself in the offspring by a reduction of approximately twenty per cent. in the average birth weight, by an increased number of deaths in the first week of life and by a general retardation in development such that the offspring of lead-poisoned males often remain permanently underweight. The testes of these chronically lead-poisoned guinea pigs have now been examined in sufficient numbers to warrant a preliminary report.

In a majority of the cases examined no histological differences can be detected between the testes of lead-poisoned guinea pigs and those of normal controls. Spermatogenesis appears to take place along normal lines and at a rate which seems not unlike that in normal pigs. This observation is in no degree incompatible with a state of true blastophthoria since it is to be expected that if fertilization takes place at all, the spermatozoa will show no great variation from the normal as far as their appearance is concerned. The reason for the inferiority of the offspring is to be sought rather in qualitative changes in the germ plasm.

In a limited group of instances, in which the male guinea pigs became sterile during the administration of lead, histological examination of the testes shows a complete aspermatogenesis with marked atrophy and vacuolar degeneration of the germinal epithelium. There is an attempt at cell division which results in multinuclear giant cells, evidently comparable to spermatocytes, but division of the protoplasm appears to lag behind that of the nuclei and spermatids and spermatozoa are not produced.

11 (1189)

The action of xanthin and methyl xanthins on the isolated intestine.

By WILLIAM SALANT and E. W. SCHWARTZE.

[*From the Pharmacological Laboratory, Bureau of Chemistry, Washington, D. C.*]

The tests were made with different concentrations of xanthin and its derivatives dissolved in Locke's solution which were carried out on segments of different parts of the intestine of the rabbit. Sodium xanthin in concentrations of 1 : 2.000 and 1 : 1.000 caused increased tonus which was more pronounced in the large than in the small intestine. The rhythmic contractions also improved under these conditions but this effect was not constant. Experiments with solutions of 1 : 10.000 sodium xanthin were negative or produced a slight stimulating action. All the methyl derivatives when employed in concentrations of 1 : 2.000 and 1 : 1,000 produced marked depression of the rhythmic movements and of tonus. The effect of higher concentrations varied. A solution of 1 : 10,000 caffein caused moderate stimulation of the movements of the small intestine but had no effect on the large intestine. This concentration of theobromin proved to be a much greater stimulant while theophyllin 1 : 10.000 induced irregular action in the small intestine with lowered tonus and disappearance of contractions in the large intestine. In still greater dilutions, however, as 1 : 50.000 theophyllin produced a marked increase in the force of the contraction of the small intestine. Similar results were obtained with 1 : 25.000 theobromin, but tests with 1 : 25,000 caffein proved to be negative.

12 (1190)

The action of succinate, malate, tartrate and citrate on the isolated intestine.

By WILLIAM SALANT, C. W. MITCHELL, and E. W. SCHWARTZE.

[*From the Pharmacological Laboratory, Bureau of Chemistry, Washington, D. C.*]

Segments of the isolated intestine of the rabbit suspended in Locke's solution and containing sodium succinate exhibited increased activity in concentrations of *N*/30 to *N*/200. Stimulation was also observed in *N*/10 succinate, but this was usually preceded by primary depression. Sodium malate in concentrations of *N*/10 and *N*/30 caused depression, the rhythmic contractions disappearing almost entirely for a period of several minutes. This was followed, however, by improvement while the intestinal segments were still in contact with the salt. Stimulation was observed in tests with *N*/70 to *N*/100 sodium malate. Sodium tartrate dextro in Locke's solution produced the following results: A solution of *N*/10 made up by adding the salt to Locke's solution caused promptly a drop of tonus; rhythmic contractions became weak or disappeared entirely for a few minutes. The fact was less marked, however, with the same concentration of tartrate when it was substituted for an equivalent amount of sodium chloride. In this case a moderate decrease only of amplitude without a change of tonus in the small intestine was noticed. The large intestine showed a marked decrease of tonus and complete disappearance of rhythmic contractions. With weaker concentrations of tartrate, such as *N*/20 to *N*/50 a decrease of tonus was obtained, which was greater in segments of the large intestine, the effect diminishing with increase in dilution. The rhythmic contractions were usually augmented in force, especially in the ileum, the effects being the same with isosmotic and with hypertonic solutions.

Observations on the action of citrate indicated that a solution of *N*/400 may increase the force of the contractions and sometimes also the tonus. The effect was different with more concentrated

solutions. Tonus was depressed in practically all cases. Although the amplitude of the rhythmic contractions showed considerable augmentation with a solution of $N/200$, this was often preceded by a preliminary decrease. Total inhibition of activity was first observed with solutions of $N/50$.

13 (1191)

The nature of the toxemia of intestinal obstruction. Preliminary report.

By L. R. DRAGSTEDT, J. J. MOORHEAD and F. W. BURCKY (by invitation).

[*From the Hull Physiological Laboratory of the University of Chicago.*]

Confirming the results of previous investigators we found that dogs with an isolated closed loop of duodenum or jejunum die in 48–96 hours, in most cases with perforation of the isolated loop and general peritonitis. But there is usually no excessive vomiting and hence no fatal dehydration of the body tissues.

In twenty-five dogs a segment of the jejunum was isolated, washed with ether and sterile water, or sterile salt solution, and both ends closed. Sixteen of the dogs died in 4–6 days, all of them showing perforation of the loop and general peritonitis. The other nine dogs lived indefinitely (some of them to date, 6 months) in good condition. Some of the dogs were examined 1–3 months after the operation. In every case, except one, the loops were found closed, the mucosa normal, some thick fluid in the lumen of the loop containing *B. coli* and a small coccus. In one dog in good condition examined seven weeks after the operation the loop was found perforated, but there was no peritonitis and the fluid contents of the loop was sterile.

When the isolated and closed loops of the jejunum is sterile complete occulusion of the blood vessels to the isolated loop has no effect on the dog, but if the loop is not sterile, the occlusion of the circulation in the loop causes death in 24–48 hours with the usual symptoms of complete intestinal obstruction.

In nine dogs a segment of the lower duodenum was isolated,

washed with ether and sterile salt solution, and the ends closed. All of these dogs died within 24–48 hours, with the usual symptoms of toxemia. In all cases the loops, on autopsy, were greatly distended, and black, or covered with purple blotches. Four of the loops had perforated. In every case the fluid in the loops contained *B. coli*, other baccilli, and cocci. The failure to get dogs thus operated to live on in good condition appears to be due to the occlusion of the circulation in the loops by distension from the duodenal secretion, subsequent necrosis, and bacterial toxemia.

In nine dogs the isolated segment of jejunum was washed with 70 per cent. alcohol or 2 per cent. lysol and sterile water or salt solution. All the dogs died within 4–18 days with perforation of the loop and peritonitis.

In four dogs a segment of the upper jejunum was washed in sterile water and replaced in the abdominal cavity without closing the ends. These dogs lived indefinitely without showing any symptoms. Five weeks after the operation a second laparatomy was performed on two of the dogs and it was found that both ends of the loops were closed by adhesions. The mucosa of the loop was normal and the fluid in the loops sterile.

In six dogs an isolated segment of the duodenum (just below the posterior pancreatic duct) was replaced in the abdominal cavity without washing the lumen or closing the ends. Three of these dogs died within five days of general peritonitis. The other three dogs lived indefinitely without showing any symptoms. One dog was inspected 20 days, and another 30 days after the first operation. In both cases the ends of the loops were completely closed by adhesions, the lumen somewhat distended with a sterile fluid, and the mucosa and muscularis normal. It is clear that the duodenal and jejunal secretions are not toxic when poured directly into the abdominal cavity.

The experiments to date, comprising work on 96 dogs, seem to warrant the following conclusions:

1. Closed intestinal loops in which the bacteria are first removed are not incompatible with life.

2. Closed intestinal loops in which bacteria are present but in which tissue necrosis is prevented are not incompatible with life.

3. Closed aseptic intestinal loops in which the blood supply is completely shut off are not incompatible with life.

4. Bacterial activity plus the necrotic tissue or the results of the action of bacteria on necrotic tissue is the important factor in the rapid death in simple closed intestinal loops.

5. The normal secretions of the duodenum and jejunum are not toxic when allowed to drain into the abdominal cavity.

6. Our results do not support the theory of Draper of a normal toxic secretion of the duodenal mucosa, neutralized by the jejunal mucosa, or the perverted secretion theory of Whipple.

14 (1192)

The effect of intravenous injections of fresh human serum and of phosphated blood, on the coagulation time of the blood in hereditary hemophila.

By THOMAS ADDIS.

[*From the Laboratory of the Medical Division of Stanford University Medical School, San Francisco.*]

The coagulation time of the blood in hereditary hemophilia fluctuates in an irregular manner from day to day. Only very pronounced alterations are therefore of value as a guide to the effect of any particular method of treatment. The variations shown in Table I were observed in cases who were not subjected to any treatment. In many instances the changes observed are well beyond the error of the method which was used. Five cubic centimeters of blood were withdrawn from the median basilic vein through a short oiled needle into two or more test-tubes, and the average interval of time required until coagulation had advanced sufficiently to allow of the complete inversion of the tubes without spilling the contents was taken as the coagulation time. The temperature was 37° C. Normal blood requires about 13 minutes to coagulate under these conditions. Parallel observations with another method showed that reliable results could be obtained with blood from skin puncture when certain details in the manner of collecting the blood were observed.[1]

[1] Addis, T., *Edin. Med. Journ.*, 1910, V, 38.

TABLE I.

VARIATIONS IN THE COAGULATION TIME OF HEMOPHILIC BLOOD OCCURRING IN CASES NOT SUBJECTED TO ANY TREATMENT.

Case I.		Case II.		Case III.	
Date.	Coag. Time. Minutes.	Date.	Coag. Time. Minutes.	Date.	Coag. Time Minutes.
Aug. 21	60	Sept. 19	60	Sept. 15	38
" 22	53	" 22	87	" 18	54
" 23	50	" 23	70	" 20	80
" 24	67			" 23	52
" 25	66				
" 26	68				
" 30	88				
" 31	96				
Sept. 4	67				
" 5	81				
" 10	55				
" 11	71				
" 19	65				
" 23	76				

Variations in coagulation time which were not greater than those illustrated in Table I were noted under the administration by mouth of calcium lactate, sodium chloride and of large quantities of raw beef juice. The removal from a vein of 60–70 c.c. of blood once a week had no effect.

No immediate effect was produced by the intravenous injection of normal horse serum and of antidiphtheritic serum. These sera were all five or more weeks old. In one case a marked prolongation of coagulation time was found three weeks after an intravenous injection of horse serum, but since successive observations were not made during this interval, it is not certain that this was the result of the serum injection.

The intravenous injection of 15 c.c. of human serum separated from freshly drawn blood ninety-six hours previously was followed within 15 minutes by a striking decrease in the coagulation time. There was a gradual increase in the coagulation time during the next week and on the tenth and twelfth days after the injection, the coagulation time was considerably longer than before the serum was given. Three weeks later the coagulation time was again at its usual level. The details of this experiment are given in Table II. The method of skin puncture was used.[1] Each figure is the average of six estimations.

TABLE II.

VARIATION IN THE COAGULATION TIME OF CASE I FOLLOWING THE INTRAVENOUS INJECTION OF HUMAN SERUM, NINETY-SIX HOURS OLD.

Date.		Coagulation Time. Minutes.	Remarks.
Feb. 24		72	Normal coagulation time 10 min.
Feb. 26		89	
Feb. 28		72	
Mar. 2	3 P.M.	62	
	4 P.M.		15 c.c. human serum 96 hours old injected intravenously.
	4:15 P.M.	24	
	6:00 P.M.	27	
	8:00 P.M.	33	
Mar. 5	2:00 A.M.	34	
	12 Noon	39	
Mar 6		40	
" 7		49	
" 10		59	
" 12		77	
" 14		127	
" 16		114	
" 17		103	
" 20		94	
" 22		86	
" 24		83	

The intravenous injection of freshly drawn whole blood to which sodium phosphate had been added as an anti-coagulant,[1] had the immediate effect of making the coagulation time ten times shorter. But here again as after the injection of fresh serum, this decrease was succeeded by a gradual lengthening of the time. Unfortunately it was not possible to continue the observations beyond the eighth day, so that it is not known whether there was a later increase in the coagulation time beyond the time required before the injection. These results were obtained with the method of venous puncture. They are given in Table III, which also shows another experiment, illustrating the immediate reduction in coagulation time caused by fresh human serum.

The recalcified oxalated plasmata prepared from blood drawn from Case 1 and Case 2 after the injection of fresh human serum coagulated in a considerably shorter time than the recalcified plasmata prepared from blood drawn before the injection. The rate of formation of thrombin was more rapid after serum injection

[1] One part of 5 per cent. sodium phosphate to three parts of blood.

TABLE III.

VARIATION IN THE COAGULATION TIME OF CASE I FOLLOWING THE INTRAVENOUS INJECTION OF ABOUT 300 C.C. OF FRESHLY DRAWN HUMAN PHOSPHATED BLOOD.

Date.	Coagulation Time. Minutes.	Remarks.
May 16	245	Normal coagulation time—13 min.
" 20 11 A.M.		About 300 c.c. of human phosphated blood injected intravenously.
12 Noon.	24	
" 22	30	
" 24	32	
" 28	55	
June 14 10 A.M.	200	
10:15 A.M.		8 c.c. of human serum 20 hours old injected intravenously.
12 Noon.	38	

than before. Smaller amounts of normal plasma were required to reduce the coagulation time of the recalcified plasma to normal after serum injections than before. It was concluded that the serum injection had altered the pro-thrombin content of the blood.

These observations were made in 1910 in the Laboratory of the Royal College of Physicians in Edinburgh during the tenure of a Carnegie Research Fellowship. It was hoped that they might be completed and extended before publication, but this has not been possible. They are of interest in connection with a recent report by Ottenberg[1] on the effect of citrated blood and of citrate solution on the coagulation time in hereditary hemophilia.

CONCLUSIONS.

1. The intravenous injection of fresh human serum causes an immediate shortening of the coagulation time of the blood in cases of hereditary hemophilia. There is then a gradual lengthening of the time until it is considerably longer than before the injection, and finally a return to the original level.

2. The intravenous injection of fresh whole blood prevented from coagulating by the addition of sodium phosphate caused a similar immediate decrease, and was followed by a gradual increase in the coagulation time during the subsequent eight days.

3. An alteration of the pro-thrombin content of the plasma-

[1] Ottenberg, R., PROC. SOC. EXP. BIOL. AND MED., 1916, 13, 104.

was found to be the cause of the increased coagulability of the blood after intravenous injections of fresh normal serum.

15 (1193)

Notes on the occurrence of equine sporotrichosis in Montana and the "blastomycotic" form of Sporotrichum schencki-beurmanni.

By K. F. MEYER.

[*From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.*]

In 1915 I[1] expressed the belief, based on very inadequate material, that animal sporotrichosis is found also in Montana. Quite recently, through the courtesy of Doctor DuFrene, of Glendive, fresh pus collected from a case of equine sporotrichosis was forwarded to me for diagnosis. Without the least difficulty a typical *Sporothrix schenki-beurmanni* was isolated on Sabouraud medium, and conclusive bacteriologic evidence was thereby obtained that sporotrichosis exists endemically in Montana.

As is customary in our studies on fungi, plain one per cent. glucose agar was inoculated with the pus. The growth on this medium remained perfectly white and thin, becoming thick, moist, very stringy and inelastic in contrast to the typical well-pigmented folded film observed on Sabouraud's agar. The culture did not penetrate into the superficial layers of the agar, and was easily emulsified. It grew well under anaerobic conditions, and produced a rapid septicaemia in rats and rabbits. On one per cent. glucose agar and plain potato, this pleomorphism has remained so far (three weeks and four transplants) constant, but on Sabouraud medium the typical growth invariably appeared in a short time.

Microscopically, such a culture consists of oblong, oval or round, short, monilia-like mycelia with a well-marked double membrane and refractile granules. Some round forms show reproduction by budding and aggregations in pairs or short chains. Long mycelia with typical clusters of spores were always absent.

[1] *Journ. A. M. A.*, 1915, LXV, p. 519.

Macroscopically and microscopically these cultures appeared in every respect like yeast or saccharomyces.

The "blastomycotic" pleomorphism has been observed by Gougerot for the *Sporotrichum beurmanni*, but has never been described for the American strains. When transplanted on very moist and soft agar, I repeatedly noticed, for at least three to four days, the yeast-like character of some human strains isolated from cases in North America, but such cultures always returned to the typical growth within ten days.

The tendency of a recently isolated strain of sporothrix to revert to its parasitic pleomorphism under unfavorable conditions (moisture, absence of oxygen, and carbohydrates) appears to be a characteristic of the European as well as of the American types, and clearly proves their close botanical relationship with the "blastomyces."

Serologic tests[1] have previously shown that complement fixation is regularly obtained with sera of infected or immunized animals when various yeasts are used as antigens.

[1] *Amer. Jour. Trop. Diseases and Preventive Medicine*, 1915, III, 144.

SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

Seventy-eighth meeting.

New York Post Graduate Medical School.

Vice-president Gies in the chair.

16 (1194)

Structure of antineuritic hydroxy pyridines.

By **ROBERT R. WILLIAMS** (by invitation).

[*From the Department of Agriculture, Bureau of Chemistry, Washington, D. C.*]

Experiments already reported have shown that hydroxy pyridine exists in two readily interconvertible desmotropic crystalline forms, one of which is able promptly to dissipate the acute symptoms of polyneuritis gallinarum. In order to ascertain the chemical structure of the physiologically active modification curative tests have been made with β hydroxy pyridine, α methoxy pyridine, α methyl pyridone, trigonelline, nicotinic acid, and betaine. On the basis of the results it may be concluded with reasonable certainty that the relief of the paralysis by such substances is intimately connected with a betaine-like ring.

That such a structure is likewise an essential feature of natural "vitamines" has been adopted as a working hypothesis. It affords a rational explanation of the instability of natural antineuritic substances and appears to conform to other previous observations. In this connection attention is called to the fact that, on theoretical grounds, the existence of betaine-like tautomeric modifications of oxy- and amino-pyrimidines and purines is not less probable than in the case of the corresponding derivatives of pyridine.

However, this assumption as to the structure of "vitamines" does not appear to be borne out by the more or less complete failure of several of the above synthetic compounds to protect birds against polyneuritis though capable of relieving the severe symptoms when once developed. No conclusion has been reached as to the cause of this apparent discrepancy.

Future work will take the direction of a search for similar desmotropism in the pyrimidine series.

17 (1195)

Quantitative chemical studies in spinal fluids.

By **R. L. KAHN** and **JOSEPHINE B. NEAL** (by invitation).

[*From the Research Laboratory, Bureau of Laboratories, Department of Health, City of New York.*]

Quantitative determinations of total, non-protein and urea nitrogen, creatinine, creatine and sugar have been carried out on spinal fluids of poliomyelitis and various forms of meningitis. Traces of ammonia, uric acid and cholesterol have also been demonstrated in these fluids.

With the exception of urea, which has been extensively studied by French workers,[1] quantitative studies in spinal fluids are comparatively meager, due undoubtedly to the fact that until recently, micro-methods not being available for these determinations, large quantities of fluid had to be used for any single chemical test.

The direct Nesslerization method for nitrogen determinations recently reported by Folin and Denis[2] has been with slight modifications adopted for the determinations of total, non-protein urea and ammonia nitrogen in spinal fluids. The total nitrogen in poliomyelitis is in the neighborhood of 25 mgm. per 100 c.c. In various forms of meningitis the total nitrogen was found to be considerably increased, extending from about 35 mgm. to 150 mgm. per 100 c.c. The non-protein nitrogen content is about 50 to 70 per cent. of the total nitrogen and urea, about 60 to 80 per cent. of the non-protein nitrogen. The determinations of am-

[1] Soper, W. B. and Granat, S., *Arch. Int. Med.*, XIII, 131, 1914, review the literature.

[2] Folin, O. and Denis, W., *J. Biol. Chem.*, XXVI, 473, 1916.

monia were unsatisfactory because sufficiently large quantities of a single fluid required for a test were not available. Mixed water-clear sterile fluids obtained from poliomyelitis cases, were used. The results range from 0.1 mgm. to 0.9 mgm. of ammonia nitrogen per 100 c.c.

For the determinations of creatinine and creatine the Folin and Denis methods were employed. These methods have been recently criticized by McCrudden and Sargent.[1] The results nevertheless seem worth reporting in view of the creatinine and creatine studies on blood with the same methods. About half mgm. creatinine and from 0.3 to 0.7 of creatine were found in fluids examined.

Sugar was determined by means of the Lewis and Benedict method. The findings in poliomyelitis are at a somewhat lower level than that of the blood.

Uric acid also was determined on mixed fluids, the results indicating that measurable amounts are present in this fluid.[2]

Attempts to determine cholesterol by Bloor's[3] method showed the presence of traces only.

AVERAGE CHEMICAL FINDINGS IN SPINAL FLUIDS EXPRESSED IN MGM. PER 100 C.C.

Number of Cases.		Total Nitrogen.	Non-protein Nitrogen.	Urea Nitrogen.	Creatinine.	Creatine.	Sugar (Per Cent.).
2	Meningism...	15	13		.36		.07
12	Epidemic cerebro-spinal meningitis...	51 (24–120)	23 (15–33)	14 (10–21)	.44	.70	Traces
8	Tubercular meningitis	30 (25–42)	15 (13–17)	9 (7–14)	.48	.56	Traces–.06
3	Influenza meningitis	.76		.6			
45	Poliomyelitis	23 (16–34)	15 (11–24)	10 (5–20)	.44 (.35–.51)	.38 (.19–.49)	.07 (.05–.09)
	"	Ammonia Nitrogen = .58		Uric Acid = Traces –.5		Cholesterol = Traces	

The figures in parenthesis indicate the lower and upper limits.

[1] McCrudden, F. H., and Sargent, C. S., *J. Biol. Chem.*, XXVI, 527, 1916.

[2] Compare Fine, M. S., and Myers, V. C., PROCEED. SOC. FOR EXP. BIOL. AND MED., XIII, 126, 1916.

[3] Bloor, W. R., *J. Biol. Chem.*, XXIV, 227, 1916.

18 (1196)

An experimental test of the relation of sewage disposal to the spread of pellagra.

By J. F. SILER, P. E. GARRISON and W. J. MACNEAL.

[*From the Robert M. Thompson Pellagra Commission of the New York Post-Graduate Medical School and Hospital.*]

After a thorough survey of the whole community of Spartan Mills in the city of Spartanburg, S. C., an endemic center of pellagra conspicuous for the number of cases of the disease which had originated in it, the surface privies were replaced by a water-carriage system of sewage disposal in the latter part of 1913 and the first half of 1914. Subsequent to this change there has been observed a remarkable reduction in the incidence of pellagra in this community, such that during the pellagra season of 1916 only one new case has appeared among the, approximately, 2,000 residents upon the mill property and this one case originated in a house situated at the very margin of this sewered district and across the street from an unsewered house in which an old case of pellagra resided. Houses situated in the partly unsewered district adjacent to the mill property furnished several new cases of pellagra in 1916. Furthermore, many of the preëxisting pellagrins living on the mill property have suffered recurrence of the disease in 1916. The results of the experiment so far would seem to indicate very clearly that the improvement in sanitation has served to prevent the non-pellagrous population from contracting the disease, but has had relatively little influence upon the course of the disease in those who had previously contracted it. This result is quite in accord with the hypothesis announced by us in 1913,[1] in order to test which this experiment was undertaken.

The detailed discussion of this experiment may be expected to appear in the Archives of Internal Medicine as one of the present series of papers, constituting the third report of the Robert M. Thompson pellagra commission.

[1] Siler, J. F., Garrison, P. E. and MacNeal, W. J., Pellagra, A summary of the First Progress Report of the Thompson-McFadden Pellagra Commission. Presented at the Special Pellagra Conference at Spartanburg, S. C., Sept. 3, 1913; *Journ. A. M. A.*, Jan. 3, 1914, lxii, 8. *Ibid.*, The relation of methods of sewage disposal to the spread of pellagra. *Arch. Int. Med.*, 1914, xiv, 453.

19 (1197)

A preliminary report on the classification of Pneumococcus IV.

By MIRIAM OLMSTEAD (by invitation).

[*Bacteriological Laboratory of the Presbyterian Hospital, New York.*]

A study of the miscellaneous group of Pneumococci, called type IV by the workers of the Rockefeller Institute, was undertaken at the Presbyterian Hospital in connection with the investigation of post-operative pneumonia instigated by Dr. Brewer and reported by Dr. Whipple before the Surgical Section of the Academy. Merely a beginning of the study has been made and the results given here are based on agglutination reactions only, which have been so clear cut as to warrant some conclusions.

Most of the strains examined have been obtained, by mouse passage, from sputum or saliva of surgical cases before operation and may be considered normal mouth inhabitants. The others have been recovered from the sputum of post-operative pneumonia cases, of pneumonia cases in the medical wards of the hospital, of bronchitis cases, from lung cultures at autopsy, and from abscess cultures. All the strains have failed to react with serum of types I and II, for which we are indebted to the Rockefeller Institute.

Immune serum has been obtained by successive inoculations of rabbits. Only sera agglutinating their homologous strains through at least a 1 in 80 dilution have been used, most of the sera agglutinate through 1 in 160.

Strains have been tested as soon as isolated against all immune sera on hand, in equal parts of serum and culture. Readings have been recorded at the end of two hours' incubation and on the following day. Positive reactions have been confirmed by tests in dilutions of 1 in 10 to 1 in 80.

Two hundred and thirteen cultures have been tested with from 1 to 15 different sera. Owing to lack of serum comparatively few cultures have been tested with all sera. This short series of agglutination tests indicates a differentiation of pneumococcus IV strains (some parasitic, some saprophytic), into more than 12 groups, some of which have subgroups. The groups are made

up of strains that have cross-agglutinated and no interaction between the different groups has been observed. Cross-agglutination tests have been incomplete, only one immune serum being used in some groups. When other immune sera have been used, as in group *A*, the results have been the same with all, and the strains have agglutinated each other in about the same dilutions.

All two hundred and thirteen cultures were tested with group *A*, which consists of 12 strains that have been agglutinated by one or more of the three immune sera of this group. None of these strains have been agglutinated by serum of group *B*, or by serum of any other group with which they have been tested. Four of them were recovered from the sputum of post-operative pneumonia cases, but as they were not agglutinated by the patients' serum the significance of their presence in the sputum is uncertain. The other eight members of the group were from normal mouths.

Two hundred and eight strains have been tested with serum of group *B* (one strain), and ten have been agglutinated. One strain was recovered from the sputum of a post-operative pneumonia case and was agglutinated by the patient's serum, one was from a bronchitis case, the other eight were from normal mouths. Another strain from a post-operative pneumonia case, agglutinated by the patient's serum and originally agglutinated by serum of group *B* in a 1 in 40 dilution later lost its agglutinability for this group. Its immune serum has no effect on cultures of group *B* and a strain agglutinated by its serum is not agglutinated by serum of group *B*. Two other instances of this sort suggest the possibility of a change in agglutinative properties, though other explanations may be offered.

Of one hundred and six cultures tested against serum of group *C*, seven have been agglutinated, one from a post-operative pneumonia case, one from a thyroid abscess, the other five from normal mouths.

Ninety-seven cultures have been tested with serum of group *D*, and five agglutinated, two from post-operative pneumonia, one from pneumonia, and two from normal mouths.

One hundred and seventeen cultures have been tested with serum of group *E*, and four agglutinated, one from post-operative pneumonia not agglutinated by the patient's serum, and three from normal mouths.

With one serum of group *F*, a strain from a pneumonia case, forty-nine cultures have been tested and only the homologous strain has been agglutinated in a high dilution, that is, through 1 in 160. Three other strains agglutinated by this serum (two from normal mouths, and one from a lung culture of a lobular pneumonia case at autopsy) formed a subgroup, agglutinated through 1 in 20 only by this serum, through 1 in 160 by immune serum of one member of the subgroup. The latter has no agglutinative action on the type cultures even 1 in 2.

Of one hundred and five cultures tested against one serum of group *G*, three have been agglutinated, two of them from post-operative pneumonia cases, and one from a normal mouth. One of the post-operative pneumonia strains was agglutinated by the patient's serum.

Immune sera of groups *H*, *I*, *J*, and *K*, have been on hand only a short time, but although few tests have thus far been performed with these strains, two members of each group have been found.

L represents a single strain, recovered from the sputum of a post-operative pneumonia case and agglutinated by the patient's serum. Immune serum of this strain has been tested with one hundred and fifty-three different cultures and has agglutinated the homologous strain only. This is apparently not a common mouth inhabitant.

CONCLUSIONS.

Pneumococcus IV strains isolated from pneumonia sputum and normal mouths seem to fall into a large number of groups. However, a considerable proportion of them are classifiable. Twelve fall into one group, ten into another, seven into another, five into another. Several other groups of from two to four have appeared and it is probable that further tests will result in an enlargement of these groups.

It seems possible that the agglutinative properties of strains may change.

20 (1198)

A method for the determination of the diastatic activity of the blood with some observations obtained in diabetes and other conditions.

By J. A. KILLIAN and V. C. MYERS.

[*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital.*]

The procedure introduced by Lewis and Benedict,[1] for the estimation of the sugar of the blood, is utilized in the estimation of its diastatic activity. Two 2 c.c. samples of oxalated blood are taken, and to one of these is added 1 c.c. of 1 per cent. soluble starch solution. Both tubes are now made up to 10 c.c. and incubated at 40° C. for 15 minutes. About 0.5 gram of dry picric acid is now added and the mixture stirred. When the proteins are precipitated, the tubes are centrifuged and the supernatant fluid filtered. The sugar in three cubic centimeter portions of the filtrates is now estimated according to the technique described by Myers and Bailey.[2] Correction is made for the sugar originally present in the blood (with the aid of the control) and for the slight reducing action of the soluble starch. The results are recorded in terms of the percentage of the soluble starch (10 mg.) transformed to reducing sugars (calculated as glucose) by the 2 c.c. of blood employed. It is believed that under the above conditions, the possible error of glycolysis is a negligible one.

The diastatic activity of the blood, according to this method, appears to vary from 15 to 25 in a variety of miscellaneous conditions in the human subject, while in diabetes, figures from 30 to 70 have been observed. The possible significance of these observations we are not prepared to discuss at the present time.

[1] Lewis and Benedict, *Jour. Biol. Chem.*, 1915, XX, 61.

[2] Myers and Bailey, *Jour. Biol. Chem.*, 1916, XXIV, 147.

21 (1199)

Studies in experimental nephritis.

By N. UMEDA and A. I. RINGER.

[*From the Chemical Laboratories of the Montefiore Hospital, New York City.*]

It was shown by Underhill and his collaborators and was corroborated by Pearce and Ringer, that the administration of tartaric acid to animals produces a very marked nephritis.

The object of our present research was to find out how the tartaric acid, which is so closely related to chemical substances that undoubtedly play a rôle in intermediary metabolism, can give rise to nephritis.

Tartaric acid does not pass through animal membranes or animal cells very readily. This is known from the fact that the salts of tartaric when given by mouth, are but slightly absorbed, and act as cathartics. We, therefore, reasoned that, since the kidney cells are more permeable to salts than are the ordinary cells of the body, and since tartaric acid forms a salt with calcium which has a very low degree of solubility, that, in all probability, the tartaric acid, while passing through the kidney cells, combined with the calcium salts of the kidney cells, forming an insoluble calcium tartrate, which becomes precipitated in the body of the cell, and thus caused the complete destruction of the cell.

If the line of our reasoning is correct, then, the administration of oxalic acid should be followed by a similar destruction of the kidney tissues, and this is exactly what we found.

In a series of animals one gram of potassium oxalate was administered subcutaneously. The animals lived for from 24 to 48 hours, during which time the blood changes were studied. After death, autopsy was performed and microscopical examination of the organs was made by Dr. B. S. Klein, of the pathological laboratory of the Montefiore Hospital.

From his reports we may draw the conclusion that oxalic acid in small quantities produces a nephritis similar to that of tartaric acid.

The conclusion, therefore, seems reasonable that tartaric acid and oxalic acid exert their influence on the kidney tubules by precipitating the calcium salts in the cells, during the process of their excretion.

22 (1200)

Do lecithin and glucose combine to form a true chemical compound?

By ERNEST L. SCOTT.

[*From the Department of Physiology, Columbia University, New York.*]

About a year ago I[1] reported a substance found after the evaporation of an emulsion of lecithin and glucose. This material seemed to be identical with the one previously reported by Bing[2] and others, the only point of difference being that previously it had been prepared by the evaporation of an alcoholic solution of lecithin and glucose upon the water bath while I evaporated a watery emulsion either on the water bath or in a vacuum desiccator at room temperature.

In attempting to study by the freezing-point method any changes in molecular weight which might occur, I found that some reaction took place between the benzene, which was used as a solvent, and the solute, lecithin, so that the results were entirely irregular and of no value for my purpose.

A further search for a solvent which could be used for the determination of either the freezing or the boiling point has revealed the same difficulty for a number of other solvents. In their turn ether, chloroform, carbon tetrachloride, bromoform, ethylene dibromide, acetic acid, formic acid and phenol were tried and discarded.

However, when the boiling point of a solution of "lecithin" in alcohol was determined the molecular weight of the sample of "lecithin" which I was using was consistently found to be 1,300. The high figure found would indicate an association of two mole-

[1] Scott, E. L., *Am. Journ. of Physiol.*, XL, p. 145, 1916.

[2] Bing, H. J., *Skand. Arch. f. Physiol.*, XI, pp. 166–175, 1901.

cules in the alcoholic solution. Since this "lecithin" was prepared by precipitation with acetone it probably contained considerable kephalin and consequently had for a single molecule a lower average weight than the 800 usually given (see Maclean[1]).

There was, as perhaps might be expected, a rise in the boiling point when small amounts of glucose were added to a solution of lecithin in alcohol. The present point of interest is that this rise was only one half as much as it would have been had no lecithin been present, provided that the sugar was added in such quantities that there was considerable excess of lecithin molecules over the sugar molecules present.

The simplest interpretation that can be placed upon this is that a portion of the lecithin disassociates and one molecule of lecithin combines with one molecule of glucose. If the number of moles of sugar added approach the number of moles of lecithin present less than half of the sugar moles disappear. This may mean either that there must be an excess of lecithin for the maintenance of equilibrium or that the sugar unites with only one of the components of the "lecithin" which as indicated above is confessedly a mixture. Again, when a comparatively large excess of sugar is added there is apparently another compound found in which more sugar is combined. This compound might be represented by L_nG_m in which m is greater than n. In the course of this work a paper by Kornfeld[2] has come to my notice in which the author has made use of the same method for the study of the hydrates of pyridine.

Again in two experiments in which a solution of the lecithin-glucose preparation was dialyzed for several days through a rubber membrane against chloroform, a reducing substance was found outside the membrane. This passage of a reducing substance did not take place when either ether or benzene was used as solvent.

Although I feel that the results already obtained throw considerable doubt upon the theory that this substance is an adsorption compound, work is being continued in which the samples of lipins used are separated and purified by the best methods available.

[1] Maclean, H., *Biochem. Journ.*, IX, pp. 351–378, 1915.

[2] Kornfeld, G., *Monatshefte f. Chemie*, pp. 865–897, 1915.

I wish to take this opportunity to express my appreciation of the kindness of Professor J. L. R. Morgan of the department of chemistry, Columbia University, who has given me the facilities of his laboratory and has freely helped me with advice and criticism.

23 (1201)

The postural activity of the rectus abdominis muscle of the cat.

By F. H. PIKE and HELEN C. COOMBS.

[*From the Physiological Laboratory of Columbia University.*]

If we accept Sherrington's[1] view that a muscle may undergo changes in length without concomitant changes in tension as a means of preserving a certain posture or attitude of the body, we find that the rectus abdominis of the cat manifests this property in a high degree.

The animals used for experiment were etherized and a tracheal cannula inserted. The skin was incised in the median line of the thorax. The pectoral muscles of one side were then severed close to their attachments to the sternum and sternal portions of the ribs and reflected outward. The tendinous insertions of the rectus abdominis on the ribs were divided and the free upper end lifted out. A thread was tied about the tendinous end of the muscle and led through a system of small pulleys to a muscle lever. The abdominal wall was kept intact. A rise of the writing point of the lever indicated a shortening of the muscle, while the writing point fell when the muscle relaxed. The thoracic and abdominal respiratory movements were recorded by Verdin tambours connected to Crile stethographs. Small changes in the length of the rectus abdominis occurred during ordinary respiration. But if fluid, usually an *M* 8 solution of sodium chloride, was introduced into the stomach through a stomach tube passed down the esophagus, or directly into the peritoneal cavity through a hypodermic needle, the muscle promptly relaxed, the amount of relaxation being proportional to the amount of fluid introduced, and continuing until the limit of distension of the abdominal cavity was reached. This limit of distension is determined by

[1] Sherrington, *Brain*, 1915, Vol. 38, pp. 191–223.

the muscular wall and not by the skin. The relaxation occurs when fluid flows in at pressures of only two or three centimeters of salt solution, and a contraction occurs when fluid is flowing out of the stomach.

The active contraction or relaxation of the rectus abdominis in response to changes of volume of the stomach or changes in volume of intra-peritoneal fluid ceased on section of the dorsal root fibers of the spinal nerves supplying the muscle, and after transection of the spinal cord at the level of the lower cervical roots. Some change in the position of the writing point of the muscle lever occurred when the stomach was distended after each of these procedures, and even a few minutes after death, but the magnitude of the changes was much less than when the central nervous system and the afferent channels were intact. Bilateral vagotomy had no marked effect on the response of the muscle.

We regard the change in length of the rectus abdominis as a necessary result of the operation of a mechanism for maintaining the relative constancy of intra-abdominal pressure. Without such a compensatory change in length of the abdominal muscles, troublesome circulatory and visceral disturbances would arise when a change in the volume of the abdominal contents occurred. Food could not enter the stomach at such low pressures as have been reported without a corresponding change in the abdominal volume.

24 (1202)

A simple method of detecting the circulation of antigen in the blood. (Preliminary note.)

By J. BRONFENBRENNER and M. J. SCHLESINGER.

[*From the Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Penna.*]

Experience with different immunity reactions has brought in the last few years a realization of very important limitations of their usefulness. It is generally conceded that it is impossible by means of immunity reactions to differentiate between actual infection and the state of immunity following it. This difficulty

was especially brought out in the beginning of the present war, when it became necessary to differentiate between the soldiers who gave positive Widal reactions due to previous prophylactic vaccinations, and those actually infected, or those who may be carriers. It was suggested by German authors that the complement deviation test may solve this problem in so much as, in their experience, artificial immunization by means of a vaccine, although followed by the development of agglutinins, did not seem to influence the production of the complement-fixing antibody in any marked degree. This suggestion was followed up in many laboratories abroad as well as in this country. In our own laboratory, for instance, this question was studied during the last two years by Doctors G. C. Simpson and J. R. Johnston, but the conclusions drawn were not very encouraging. The necessity of differentiating between actual disease and the state of immunity is not limited to the case of typhoid. Since it has become the practice in large institutions to apply the Shick test, it has also become necessary to differentiate between individuals containing antitoxin in their blood as a result of natural immunity and those who may be harboring a mild infection or those who may be carriers. In case of diagnosis of gonnorhea many workers have noticed that often very old cases without any symptoms of the o'd infection for many years, still frequently give a positive complement deviation test. The same is true for tuberculosis, syphilis and, in fact, for any infectious disease, for antibodies are known to persist in the blood for a certain length of time after the actual recovery from the infection. It is evident that if it were possible in addition to detection of antibody in the blood to detect also the antigen, where it is present, it would permit one to differentiate the condition of disease in its incubation period or in its mild course, from the condition of immunity, following the disease or artificial immunization.

Recently, in the midst of other work, which will follow later, we have made an observation which seems to indicate this possibility. We noticed that in certain stages of different infectious diseases the antigen and antibody coëxist in the blood and may cause the fixation of complement. The fluctuations in the complement content of the blood were noticed long since, but the

observers failed to find any laws governing these fluctuations, and therefore did not attribute any diagnostic significance to this phenomenon. As any antigen and its corresponding antibody will cause fixation of the complement of the blood, it i evident that the reaction is not specific and could not be used instead of accepted methods for diagnosis. However, if this reaction is used in addition to other tests it gives very valuable information, Thus, for instance, in the cases of syphilis treated with salvarsan. the Wassermann reaction may remain positive, whereas our test gives negative reaction as soon as the antigen disappears from the blood. In cases of gonnorhea of many years standing, we obtained negative reactions, whereas the cases of short duration or with discharge at present gave positive reactions.

Thus, the negative outcome of the test seems to be of great value, especially in the face of positive findings by usual methods. Since the positive outcome of this test may be influenced by many different conditions, we hesitate at present to attribute to it any more value than that of a very promising suggestion. We hope, however, to be able by isolating the antigen from its combination with antibody in the blood, to make also the positive phase of the test of more value in determining the circulation of antigen.

25 (1203)

The new-formation of hemal nodes in the omentum and mesentery of the dog after splenectomy and ligation of the splenic veins. (Preliminary report.)

By ALDRED SCOTT WARTHIN, PH.D., M.D.

[*From the Pathological Laboratories of the University of Michigan, Ann Arbor.*]

The question of the new-formation of splenic or hemolymph-node tissue in the dog after splenectomy has been opened up again by Meyer,[1] who, as the result of the findings of eight dogs examined after splenectomy, at periods of 30, 41, 53, 77, 89, 98, 112 and 126 days after the operation, found no changes in the lymph-nodes, either of the nature of a hyperplasia or regeneration. Doubt was,

[1] *Journal of Experimental Zoölogy*. 1914.

therefore, cast upon the work of preceding investigators, notably that of Tizzoni.

During the years 1910–13 several series of experimental investigations in regard to this point were carried out in my laboratory, with results as yet unpublished. These are now presented here in the form of a preliminary report.

Results of Splenectomy.—Nine dogs were splenectomized and examined as follows: Three one week after the operation; three two weeks after, and three one month after. In no dog was there the slightest change apparent in the lymph-nodes of any part of the body.

Five dogs were splenectomized and examined eight months after the operation. In one dog the lymph-nodes and hemolymph-nodes were larger than they had appeared to be at the time of operation when these nodes were examined as carefully as they could be. No new-formation of hemolymph-nodes was seen.

Five dogs were splenectomized and examined eight and a half months after the operation. No hyperplasia and no new-formation of lymph-nodes was seen.

Five dogs were splenectomized and examined nine and a half months after the operation. In one dog there was distinct enlargement of lymphatic and hemolymph-nodes, but no new-formation.

Results of Ligation of Splenic Veins.—In eight dogs the splenic and gastrosplenic veins were ligated as completely as possible. The animals were examined at periods of one week, two weeks, three weeks, one month, three months, one year, one year and a half, and two years after the operation. These animals were carefully examined at the time of operation for the presence of hemolymph nodes or accessory spleens in the gastrosplenic and great omentum, as well as in the peritoneum and mesentery. No changes in the lymph nodes and no new-formation of hemolymph or splenic tissue was found in the first four dogs. In the dog killed three months after the operation the prevertebral lymph nodes and hemolymph nodes appeared to be slightly enlarged. In the dog examined one year later the prevertebral nodes were much enlarged, but no new formation was noted.

In the dog examined eighteen months after ligation of the

veins the prevertebral hemolymph nodes were very hyperplastic, and the gastro-splenic and great omentums were strewn with small reddish points that microscopically present the appearance of developing hemal nodes. In the dog killed two years after the ligation very remarkable changes were found, the omentums, mesentery and peritoneum in the upper left quadrant were strewn with innumerable red nodes, varying in size from that of a pin-head to that of a pea, and in two cases, as large as a cherry. These large ones had all the gross appearances of accessory spleens; but *they had not been present at the time of the operation.* Microscopically these nodes presented all stages of transition in the development of hemal nodes, from the minute dilated lobule of sinusoidal capillaries in the fat tissue up to the fully developed hemal node resembling an accessory spleen. These nodes were precisely the same as those in the preceding case. In both cases the spleen was atrophic.

Conclusion.—Splenectomy in dogs is not comparable in its effects upon the lymph nodes to the same operation in sheep and goats. In only two out of twenty-four dogs was any hyperplasia of the lymph nodes found, and no new-formation of hemolymph-nodes or splenic tissues.

Ligation of the splenic veins seems to produce much more decided results after the lapse of one or two years following the operation. Three out of eight dogs showed marked hyperplasia of the prevertebral nodes, and the two cases examined after longest intervals showed a new-formation of hemal nodes in the splenic region, the largest newly formed nodes resembling accessory spleens. These structures were not present at the time of the operation.

They are not inflammatory in character.

Individual dogs must have different capacities for hyperplasia and regeneration. The minute capillary plexuses from which these nodes arise may represent preformed accessory spleen anlage.

Tizzoni's statements are hereby confirmed and Meyer's objections negatived.

26 (1204)

A study of the lipin-content of the liver in two cases of pituitary dystrophy. (Preliminary report.)

By ALDRED SCOTT WARTHIN, PH.D., M.D.

[*From the Pathological Laboratory of the University of Michigan, Ann Arbor.*]

Studies of the lipins in the curious obesity characteristic of hypopituitarism have not as yet been made. In the case of two autopsies upon bodies showing this condition microchemical studies have been carried out by the writer.

One case was a boy of eighteen showing infantilism, obesity and loss of sight (dystrophia adiposo-genitalis). Died after attempted removal of pituitary neoplasm (adamantino-carcinoma); the second case was a man of 29 years, with loss of vision, staggering gait and disturbed mentality. Acromegalic symptoms with secondary hypopituitarism. Round-cell sarcoma of base of skull destroying a hypophysis adenoma.

Both bodies showed very marked post-mortem increase of temperature; the source of the heat-production apparently being localized in the panniculi and fatty livers.

Gross Appearances of the Fat.—Panniculi thick, in large coarse lobules; when first cut it was glistening, translucent, very firm and light buffy yellow in color. As it cooled it became more buff in color, opaque and very hard. The livers of both cases were large, mottled red and yellow, with fatty shine.

Microscopical.—Frozen sections examined optically showed the presence of numerous small anisotropic droplets in liver, adrenals, panniculi, intima of aorta, and elsewhere. In the liver the fine anisotropic droplets are chiefly in the central and midzonal regions. They are present also in a narrow border of preserved liver cells in the peripheral zone. Larger isotropic droplets are scattered irregularly through the lobule, usually occurring in groups. There is in both cases a peculiar necrosis of the inner portion of the peripheral zone (intra-peripheral necrosis).

Staining with *osmic acid* gives a pale gray tint to the fine anisotropic droplets, while the larger fat-drops stain a deep brown-black.

Sudan III stains the fine fat droplets a peculiar brownish red; while the larger fat droplets take a deep brick-red to a yellow or pale buff color.

Scharlach R stains the fine fat-droplets a brownish-red, the larger ones a deep bright red.

Nile-blue sulphate stains the majority of the fine droplets a pink or lavender color. The majority of the larger droplets stain red, some pink, some pink-violet, some purple, some deep blue.

Indophenol. No differential staining shown.

Benda's Method. Gave no differential reaction.

Stains for fatty acids and soaps negative.

Simple staining with hematoxylin and eosin showed a typical ground-glass appearance in the cells containing the fine fat droplets.

From these fat reactions it is evident that the livers of these two cases of dyspituitarism show an unusual lipin content. The presence of numerous anisotropic droplets, staining a lighter gray with osmic acid, brownish or yellowish-red with Sudan III or Scharlach R, and pale pink with Nile-blue sulphate, makes it very probable that these are cholesterol-esters. Other lipoids may be mixed with these or with the glycerin-esters. The lipin combination may be very complex. Our staining methods do not satisfactorily differentiate these. They permit us to say, however, that these fatty changes are not those of ordinary fatty infiltration, but represent a mixed glycerin-ester and cholesterol-ester lipoidosis. It is evident that many of the liver cells contain cholesterol. Whether lecithin is present it is impossible to say.

This cholesterol lipoidosis was not confined to the liver, but occurs to a marked degree in the adrenals, and to a lesser degree in the panniculi and other organs and tissues (aorta, spleen, etc.). In pituitary dystrophia there occurs, therefore, a peculiar abnormal lipin metabolism characterized by cholesterol infiltration or retention (cholesterol steatosis). The condition may then be compared to the cholesterol infiltrations obtained by overfeeding with cholesterol, to the lipoidosis of diabetic lipoidaemia and that of Gaucher's disease.

In addition the liver shows a peculiar intraperipheral zonal

necrosis differing from previously described liver-necrosis. With this necrosis there is associated a fibro-blastic proliferation giving the picture of an early intralobular cirrhosis.

27 (1205)

The comparative effect of adrenalin on the pupil and blood pressure in cats and rabbits.

By T. S. GITHENS.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

Several years ago Meltzer showed that there was a striking difference in the response of rabbits and cats to instillation of adrenalin in the eye after excision of the superior cervical ganglion. Whereas in rabbits full dilatation was obtained by a single instillation, in cats it was obtained inconstantly and only after very numerous instillations.

In order to determine whether this difference in behavior was due to a difference in the susceptibility of the iris in the two species to adrenalin, I gave this by intravenous injection and made a quantitative study of the iris on both the normal and gangliectomized sides. I found that contrary to expectation, the iris of the cat was much more sensitive than that of the rabbit both in the intact and gangliectomized eye. Whereas 0.1 mg. per kilo, caused in the rabbit only slight dilatation of the normal pupil, this dose caused maximal dilatation in the normal pupil of the cat. On the gangliectomized side 0.01 mg. per kilo which had very little effect in the rabbit, caused almost maximal dilatation in the cat.

It was noted however that the duration differed in the opposite direction, being much greater in the rabbit. Thus a dose of 0.1 mg. per kilo, which caused dilatation lasting half an hour in the gangliectomized cat's pupil, caused dilatation lasting more than three hours in the rabbit. Smaller doses showed a similar relation.

In order to determine whether this relationship is true of smooth muscle in general or is limited to the iris, I studied the rise of blood pressure in the two species. The results showed that

with all doses the average effect of intravenous injections of adrenalin on the blood pressure was greater in the rabbits than in the cats both in regard to the height and the duration of the rise.

We may then conclude that the difference in the effect of instillations is not dependent on greater sensitiveness of the iris of the rabbit and that the greater sensitiveness of the cats' dilator muscle to the action of adrenalin is not shared by all other smooth muscle.

28 (1206)

Proteose intoxications and body protein injury.

By G. H. WHIPPLE and J. V. COOKE.

[*From The George Williams Hooper Foundation for Medical Research and the Department of Pathology, University of California Medical School, San Francisco.*]

Proteose injections in dogs cause well-known clinical reactions —vomiting, diarrhea, temperature reactions, low blood pressure, prostration and after large doses, an excess of antithrombin with incoagulable blood. A single proteose injection—for example one half a lethal dose—causes abrupt clinical reactions in a normal dog with apparent complete recovery within 24 to 48 hours. The nitrogen elimination curve in a fasting dog under such conditions shows a great rise in total urinary nitrogen. The apex of the curve usually falls on the second 24-hour period following the injection. This rise may be over 100 per cent. increase above the mean base line nitrogen level. It does not fall promptly to normal but declines slowly in 3 to 5 days or more toward the original base line. This speaks for a definite cell injury with destruction of considerable protein substance due to a single proteose injection. The disturbance of cell equilibrium is not rapidly nor promptly restored to normal.

A dog which has received previous proteose injections is somewhat immune or tolerant to subsequent injections of proteose. Such dogs, as a rule, show less intense clinical reactions and less rise in the curve of nitrogen elimination following a unit dose of standard proteose as compared with normal or non-immune con-

trols. The proteose used in these experiments was prepared as described from material obtained in cases of intestinal obstruction or of closed intestinal loops.

Dogs with isolated loops of small intestine show many evidences of intoxication. A study of the total nitrogen elimination shows a great rise above the normal base line minimum of this fasting period. This means that this intoxication is associated with a great destruction of body protein, and explains the high non-protein nitrogen of the blood which was observed and reported previously.

Dogs injected with sublethal doses of proteose will show a definite tolerance to subsequent injection, and will show much less acute intoxication after the isolation of a closed intestinal loop. Such immune or tolerant dogs show a much less pronounced rise in the nitrogen elimination curve during proteose intoxication of any type. This indicates that the tolerance or immunity to proteose gives more protection for the body proteins against the injury which these toxic proteoses inflict upon the body cells.

Complete duodenal obstruction combined with a gastrojejunostomy gives a chronic type of intestinal obstruction associated with little vomiting, which is peculiarly suited to metabolism study. Such duodenal obstructions show a definite and sustained rise in the curve of nitrogen elimination above the normal base line level. These dogs, too, are tolerant to injections of standard toxic proteoses.

Control ether anesthesia experiments show little if any rise in the curve of nitrogen elimination.

Control laparotomy experiments show a definite rise in the curve of nitrogen elimination, but a rise which is small compared with the rise noted in the intoxication of duodenal obstruction or isolated intestinal loops. It is highly probable that the tissue injury and disintegration associated with the wound reaction are responsible for the general reaction. We may assume that protein split products from the wound area are absorbed, and are responsible for the general reaction observed.

Metabolism studies on fasting dogs show that during the intoxication accompanying the formation of a subcutaneous abscess there is a marked increase in nitrogen elimination. This

increase is still further augmented for a short period after the abscess is incised, but during healing gradually returns to normal. This increased excretion of urinary nitrogen is accompanied by diuresis.

Acute pancreatitis from injection of bile in the pancreatic duct shows a similar metabolic disturbance.

Studies on fasting dogs suffering from acute pleuritis, pneumonia, acute endocarditis and distemper show that marked increase in nitrogen excretion is a constant phenomenon in these inflammatory processes. If one infection such as pneumonia is complicated by another infection such as endocarditis, the rise in nitrogen elimination is still further augmented.

In the above conditions, the blood non-protein nitrogen is increased, although the blood urea tends to remain relatively low.

These phenomena accompany acute inflammatory lesions caused by bacteria and also sterile lesions induced by an irritant (turpentine).

From the exudates in acute purulent inflammations toxic proteose-like substances have been isolated.

We wish to assume that the intoxications here studied are associated with a definite proteose intoxication, which is capable of initiating and continuing a profound injury of tissue protein. One index of this protein injury is the great and sustained rise in the curve of total nitrogen elimination.

29 (1207)

Botulism.

By ERNEST C. DICKSON.

[*From the Division of Medicine of Stanford University Medical School, San Francisco.*]

In a previous report[1] it was shown that the formation of toxin by the *Bacillus Botulinus* is not dependent upon the presence of animal protein in the culture medium, but that in purely vegetable medium it may be formed with almost equal facility. The report was based upon experiments in which beans and peas were used, but later experiments have shown that corn and apricots are also suitable for the development of the toxin.

The importance of these observations has been emphasized by the fact that within a few months there have been three outbreaks of botulism with eight deaths in which the cause of the poisoning was the ingestion of home-canned beans, corn and apricots, respectively. In all cases a number of chickens became paralyzed and died after eating the remnants of the food which had been discarded. The virulence of the toxin was very great in all cases, that in the beans and corn being so great that the patients died after merely tasting the contents of jars in which the odor was unusual.

Records of necropsy and of histologic examination of the tissues of the chickens are not available in the corn and apricot cases, but examination of the tissues from the patient and from the chickens which died after eating the beans revealed the characteristic thromboses which were first observed by Wilbur and Ophüls[2] and which were reproduced experimentally by the author.[1]

From the contents of the crops and gizzards of the chickens which died after eating the beans and corn, an organism was recovered which is morphologically and culturally identical with the *Bacillus Botulinus*, and which produces a toxin by which the typical symptoms and the characteristic thrombosis may be reproduced in animals. The virulence of the toxin in both strains is extremely high, approximately 0.0002 c.c. of a filtered beef infusion culture of the bean strain being sufficient to kill a small guinea pig within eighteen hours, and 0.001 c.c. of a similar culture of the corn strain being sufficient to kill a medium sized rabbit within twenty hours.

[1] DICKSON, E. C., Botulism, an experimental study. A preliminary report. *Jour. Amer. Med. Assoc.*, 1915, LXV, 492.

[2] WILBUR, R. L. AND OPHÜLS, W. Botulism. A report of food poisoning apparently due to eating canned string beans, with a report of a fatal case. *Archiv. Int. Med.*, 1914, XIV, 589.

30 (1208)

The effect of pituitrin and adrenalin on the urea-excreting function of the kidney.

By T. ADDIS and G. D. BARNETT.

[*From the Medical Division of Stanford University Medical School, San Francisco.*]

When the rate of urea excretion is determined for successive periods of time of short duration (15 to 75 minutes) changes in the rate are frequently noted which cannot be accounted for by synchronous alterations in the concentration of urea in the blood.

Such a change in the rate of urea excretion may be produced in man by the intravenous injection of pituitrin (Parke, Davis & Co.). Immediately after the injection there is a decrease in the rate of urea excretion without any corresponding alteration in blood urea concentration.

In rabbits whose kidneys were placed under conditions calling for a maximal exercise of their urea-excreting function by the administration of 5 grams of urea by stomach tube, the subcutaneous injection of 0.25 c.c. of pituitrin was accompanied by a decrease in the hourly rate of urea excretion, although the blood urea concentration was not lower than in control experiments, in which no pituitrin was given.

Adrenalin (Parke, Davis & Co.) injected subcutaneously in doses of 0.5 c.c. of 1 in 1,000 solution into rabbits under the same conditions did not alter the hourly rate of urea excretion, although the blood urea concentration was not so high as in the control experiments without adrenalin.

Pituitrin therefore decreases, and adrenalin increases, the urea-excreting capacity of the kidneys.

SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

Seventy-ninth meeting.

Rockefeller Institute for Medical Research.

President Jacques Loeb in the chair.

31 (1209)

The intranuclear origin of the mammalian red blood corpuscles observed in living cultures.

By **R. W. TOWER** and **C. F. HERM** (by invitation).

[*From the Department of Physiology, American Museum of Natural History, N. Y.*]

The present experiments on the origin of the mammalian (cat) red blood cells were the outgrowth of a study to determine the different stages in the development of the normoblast into a true red corpuscle by means of a living culture of red bone marrow. It was early found that the modern explanation of the formation of the mammalian red blood corpuscle did not agree with the activities observed in the cultures and that instead of witnessing a normoblast re-modeled into a non-nucleated corpuscle by losing its nucleus we saw this same normoblast sending out unnucleated straw-colored bladders from its nucleus, these bladders finally separating off as true red corpuscles. This process was not confined to the normoblast type but was evident in the lymphocyte series of white cells. Moreover our observations show that the normoblast in the mammal (cat) and the red corpuscle in the bird (chick) arise from white cells by an intranuclear activity and at the time when they first emerge from the parent cell they are almost indistinguishable from each other.

The principal results are:

1. The mammalian red blood corpuscle is a nuclear bud which escapes into the circulation as the true red cell.

2. The mammalian normoblast and the red corpuscle of the bird are the product of intranuclear activity and are phylogenetically identical.

3. Phagocytosis of red cells by the giant cells (megakaryocytes) in normal blood-forming tissues is by no means common. The true process is undoubtedly the manufacture of red cells and not the destruction of them.

32 (1210)

The use of pancreatic vitamine in cases of infant malnutrition.

By WALTER H. EDDY and JOSEPH C. ROPER.

[*From the Department of Pathology, New York Hospital.*]

Preliminary experiments by the senior author had demonstrated that pancreatic vitamine is removed very completely by treating the containing extract with Lloyd's reagent (50 g. Lloyd to 1 liter of extract). When the activated Lloyd powder is dried it retains its power as a vitamine carrier and in case of rats exercises the same power as water solutions of vitamine.

Basing work upon the above fact, attempts were made to restore to growth marasmic children, by mixing activated Lloyd powder with their cereal. In these studies Dr. Roper assisted in selecting the children from the wards of the New York Hospital and in directing their treatment.

Experiments were begun in October and the treatment has now been applied to ten babies, including one furnished by the Babies Hospital through the kindness of Dr. Bartlett.

In all the cases the addition of the powder has been followed by increased growth of a normal tissue-forming type. In the case at the Babies Hospital this growth was the first normal increase since July 25.

In one case at the New York Hospital the child was being fed on cereal and condensed milk. This case was selected for special control study. For this purpose five rats were selected

and fed with the same cereal and condensed milk as the child. The effect of adding or omitting vitamine to the diet of the rats and child are shown in the following table.

DIETARY FIGURES ON CASE FED UPON A CEREAL AND CONDENSED MILK DIET.

	Vitamine Given with Cereal at Beginning Diet.		No Vitamine Given with Cereal at Beginning Diet.			Child John G.
	Rat No. 20.	Rat No. 22.	Rat No. 16.	Rat No. 17.	Rat No. 18.	
Weight at beginning of diet	69	50	78	66	76	3,444
Weight at end of diet	88	73	62	67	76	
			Dead	Dead	Alive	3,920
No. days of diet	13	13	23	22	24	21
Net change in weight	19	23	16	1	0	*476
Total cereal consumed	446	452	768	660	792	
Ave. daily consumption	34	35	32	30	33	
Ave. daily consumption milk	56	56	56	56	56	
Second Period						
Weight of rat when vitamine was stopped	88	73	—	—	—	3,920
Weight of rat when vitamine was resumed	88	89	—	—	—	4,088
No. days elapsed	12	12	—	—	—	9
Net change	0	16	—	—	—	168
Third Period						
Weight when vitamine was resumed	88	89	—	—	76	4,088
Weight at end of period	118	113	—	—	111	4,368
No. days elapsed	11	11	—	—	11	15
Net change	30	24	—	—	35	280

33 (1211)

Influence of pituitrin and of adrenalin on the pupil of normal and ganglionectomized rabbits. A demonstration.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Reserch, New York.*]

By **T. S. GITHENS** and **S. J. MELTZER.**

The impression prevails that adrenalin and pituitrin affect the body in the same direction. The most striking action is the production of a blood pressure rise by both substances. The action of both substances upon the uterus and upon the intestines

* This gain was made on addition of 2 grains of vitamine per day.

are also in the same direction; the difference is only a quantitative one and it is slight. Several years ago one of us (M.) reported before this society that pituitrin acts strikingly different from adrenalin upon the pupil of rabbits on the side in which the superior cervical ganglion has been previously removed. We have recently studied the subject from various angles and wish to demonstrate the obtained results on these three rabbits. In two of these animals the superior cervical ganglion has been removed on one side several days ago; the third rabbit is a normal one. The demonstration shows the following facts. (1) The intravenous injection of pituitrin in a normal animal produces a considerable constriction of the pupil, while adrenalin produces a moderate dilatation of short duration. (2) The intravenous injection of adrenalin causes a maximal dilatation of long duration of the pupil on the side in which the ganglion was removed, while injection of pituitrin causes rather a constriction. (3) When adrenalin and pituitrin are injected simultaneously into a rabbit in which a ganglion is removed the corresponding pupil dilates, but the dilatation is much less than that of the pupil of a ganglionectomized animal which received adrenalin alone.

Two facts stand out clearly: First, the effect of adrenalin upon the pupil is dilatation, while that of pituitrin is constriction; second, with regard to their action upon the pupil pituitrin *counteracts* to a degree the dilating effect of adrenalin.

34 (1212)

Prolonged constriction of the bloodvessels by subcutaneous injection of adrenalin into the ear of a rabbit. A demonstration.

By JOHN AUER and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research, New York.*]

Intravenous injection of adrenin causes a rise of blood pressure which is due to a constriction of the bloodvessels. The rise lasts only several minutes. The shortness of the duration of this rise is explained by the escape of the injected adrenin from the circu-

lation into the surrounding tissues where it becomes destroyed by the alkalinity of the tissue fluid. Subcutaneous injection of adrenin produces only a slight effect upon the blood pressure. As is well known the bloodvessels of the rabbit's ears are easily visible. We have observed that a subcutaneous injection of adrenin into the lower part of one of the rabbit's ears causes a striking and long lasting constriction of the bloodvessels of the corresponding ear. It is this phenomenon which we wish to demonstrate. If the injection is given in the proximity of the central artery, the constriction of the bloodvessels of the entire ear sets in two or three minutes after the injection. If it is given at some distance from the central artery, the pallor develops slowly. In either case the vessels remain more or less strikingly constricted for many hours, sometimes even seven hours. Injection of a different solution, saline, for instance, does not exert such an effect. It is evident that in the case of the ear the adrenin is not destroyed rapidly by the tissues surrounding the bloodvessels.

35 (1213)

Colostral blood-serum therapy.

By CHARLES B. FITZPATRICK.

[*From the Research Laboratory, Department of Health, New York City, New York.*]

My observations on natural antibodies and healing substances in milk[1] led me to study the transmission of antitoxines in colostrum. Some buck kids were fed from birth until they were about one month old on the colostrum milk which their mothers gave after delivery. The blood-serum of these young goats was utilized for this study.

The ingestion of the colostrum of goats by man has disagreeable results. The greatest amount of the immunizing substances both natural and artificial which pass from the nursing mother to the blood-serum of the infant, however, is transmitted by the colo-

[1] "The Utilization of 'Reactor' Milk in Tubercle-Medicine," *Proc. Soc. Experimental Biology and Medicine*, 1915, xiii, pp. 35–37.

strum. Antitoxine taken in milk by infants appears to a large extent in their blood-serum. I reasoned that the administration of the blood-serum of these goat kids, obtained from them shortly after they had taken the colostrum, would be beneficial to infants in need of natural antibodies to overcome the hereditary dystrophy of children born of tuberculous mothers. This blood-serum I have here referred to as colostral blood-serum.

Tests of this serum were then made, and it was found to be harmless. Thirty to sixty c.c. of this colostral blood-serum were given every two to four days over a period of two weeks. These doses, each of which had been mixed with a pint of goat's milk, were fed to a scrofulous infant two months old, with a prognosis of extreme gravity. This infant was born of a tuberculous mother. The results indicated undoubtedly a marked benefit. A striking improvement took place during the two weeks following the ingestion of this blood-serum and continued thereafter, until the infant appeared to be about normal. The first dose consisted of 30 c.c. of the colostral blood-serum, used in two days, the second, of 60 c.c. fed in two days, the third of 60 c.c. in four days, the fourth of 50 c.c. in four days, and the fifth of 50 c.c. in two or three days. A total of 250 c.c. was given in about two weeks. This infant was on goat's milk from directly after birth, but did not definitely improve until after the colostral blood-serum had been employed.

The colostral blood-serum, in order to be effective in older infants, when the intestinal wall has become more impervious, and in adults, must most probably be injected, subcutaneously, intravenously or subdurally. Direct absorption of protein may, however, occur late where the condition of the alimentary canal is abnormal. Although the result was obtained in a scrofulous infant, this practical method of obtaining natural and artificial antibodies gives promise of being effective in syphilis and other diseases influenced by antibodies.

We must, besides the antibody aspect, also take the activation concept of colostrum into consideration. The mother of this infant did not furnish it with colostrum. It may well be since constituents of ingested colostrum, especially proteins, pass into the blood-serum, that by this technique we have found a way of using these important constituents and the functions of colostrum. This

method of employing the blood-serum of the colostrum-fed, furthermore, does not cause colostration.

36 (1214)

Abnormalities in the Q R S group of the electrocardiogram associated with myocardial involvement. (Preliminary report.)

By **B. S. Oppenheimer** and **M. A. Rothschild.**

[*From the Cardiographic Laboratory of Mount Sinai Hospital.*]

We have noted the association of definite peculiarities in the electrocardiogram, indicative of disturbed intraventricular conduction, with such clinical conditions as are usually accompanied by myocardial involvement.

The normal electrocardiogram is to be considered the result of the passage of an impulse at a normal velocity through the usual channels, *i. e.*, node of Tawara, main stem, bundle branches and arborizations which consist of the so-called Purkinje fibers. The latter form a network covering practically the entire endocardial surface of the ventricles. The velocity of the impulse through Purkinje fibers is at least ten times faster than its rate through ordinary ventricular musculature. The impulse reaches the ventricle normally through the Purkinje fibers, stimulating the ventricular walls practically as a whole. An experimental injury to the Purkinje fibers delays the propagation of the impulse. A lesion only partially involving either bundle branch or an extensive lesion of the arborizations of a branch would cause a delay in the transmission of the excitation wave over the area supplied or damaged. An experimental block of either right or left main branch completely interrupts the passage of the impulse over normal channels to the corresponding ventricle, the spread then occurring through ordinary muscular connections. This gives a characteristic electrocardiogram differing in many respects from those to be described here which we believe to be intermediate forms between the normal and those due to bundle branch block. It is possible that some of the clinical records interpreted as bundle

branch block may be instances of only partial blocks or rather of lesions beyond in the arborizations. Our gross pathological specimens tend to support this view.

The criteria in the electrocardiograms which we have used are in general as follows:

1. Abnormal prolongation of the time interval of the *QRS* group beyond the normal limit of 0.1 second. This prolongation is most manifest in a widening of the *R* wave, so that its foot pieces are abnormally separated. The *R* wave no longer has its slender, tall, spike-like appearance, but is broader and sometimes blunter than normally.

2. Notching of the *R* wave. This notching may appear on the ascending or descending limb, on both limbs, or at the peak. It may be multiple, and its degree and location may vary slightly from beat to beat. In arrhythmias, the shorter the preceding inter-ventricular interval, the more pronounced the evidence of disturbed intra-ventricular conduction.

3. Low voltage as expressed by a low amplitude of the waves in all three leads. This change is not uniformly present, but when it occurs it helps to differentiate this type from the electrocardiograms typical of bundle branch block.

4. Absence of the typical diphasic curves with huge *T'* waves found in experimental bundle branch block.

We have obtained records of 17 such cases which presented the clinical pictures of arteriosclerosis, coronary artery disease, angina pectoris, cardio-vascular-renal disease, syphilis, myocardial disease, some associated with auricular fibrillation or flutter, and we have learned that the prognosis of these cases is serious—probably graver than that of those individuals who present records typical of block of the right or left *chief* branches of the bundle of His.

Pathology.—In but five of the cases have we had an opportunity to examine the hearts pathologically; in a sixth case we secured only the septum including the atrioventricular bundle. Serial sections are being prepared.

1. At the present time we can report that four of the cases showed coronary artery sclerosis with closure of the anterior descending branch of the left coronary artery. This artery gives off septal branches which supply the anterior part of the septum.

(The bundle of His and its two main divisions are supplied chiefly by branches from the right coronary artery.)

2. All five cases had a widely disseminated patchy sclerosis. The one case which did not belong to the atherosclerotic group was aged twenty and had no known etiology for the interstitial myocarditis except a recent grippe with cardiac disturbance.

3. The pathological changes, especially the sclerosis, predominate in the endocardial and subendocardial layers, *i. e.*, in the region of the Purkinje network, as compared with the outer two thirds of the ventricular musculature.

4. These changes were grossly more marked in the left ventricle than in the right.

Experiments with the use of two galvanometers have been planned to test out our tentative suggestion that the above mentioned changes in the electrocardiogram are evidence of a serious conduction disturbance in the tissues beyond the termination of the right and left chief branches of the atrioventricular bundle.

37 (1215)

The occurrence of lichenase in the digestive tract of invertebrates.

By HOWARD B. LEWIS and MINNA E. JEWELL.

[*From the Laboratory of Physiological Chemistry of the University of Illinois, Urbana.*]

A reducing sugar identified as glucose by the formation of the characteristic osazone was produced from lichenin (purified carbohydrate and crude extract of Iceland moss) by the action of extracts of the hepatopancreas or alimentary canal of twenty species of invertebrates, representing the following phyla; Porifera, Annelida, Echinodermata, Mollusca, Arthropoda, and Tunicata. No evidence of a lichenin-splitting enzyme was observed in twelve species of vertebrates, embracing the following classes: Pisces, Amphibia, Reptilia, Aves, and Mammalia. The activity of the preparations tested was in every case controlled by tests for an amylase, which was invariably found to be present. Extracts of muscle tissue of the crustaceans could not split lichenin. The

constant occurrence of lichenase in the digestive tract of the invertebrates studied, suggests that the ability to hydrolyze lichenin may be a characteristic of invertebrates as contrasted with vertebrates. The presence of an inulase or raffinase in the species studied was not constant. Lichenase to judge from the present series of experiments is not invariably associated with inulase as has been suggested (existence of an inulo-lichenase). The following species were studied; sponge, earthworm, leech, starfish (2 species), sea urchin, chiton (2), mussel (3), snail (2), crab (2), shrimp, grasshopper, tunicate, gold fish, frog (adult and tadpole stages), horned toad, garter snake, terrapin (2), domestic fowl, wild rabbit, pig, sheep, dog, and man (saliva). The strongest reactions were obtained with the star fish (*Asterias ochracea*), snail (*Planorbis trivolvum*), and grasshopper (*Melanoplus differentialis*).

38 (1216)

Further studies in serum sickness.

By RICHARD WEIL, M.D.

[*From the Department of Experimental Therapeutics, Cornell University Medical School.*]

In a previous paper,[1] I showed by anaphylactic methods that the blood of human beings who had received large injections of therapeutic horse serum contained not only horse serum, but antibodies thereto, at some stage of the serum sickness. Since that time I have approached the same problem by means of the precipitation method, which is very much the more delicate method for the purpose. Remnants of horse serum in the blood are demonstrated by precipitation with the serum of a rabbit immunized to horse serum. Antibodies to horse serum are demonstrated by precipitation of horse serum by the human serum. By this method it has been possible to demonstrate the presence of horse serum in the blood from the time of injection up to more than twenty-one days thereafter, in constantly diminishing amount. Antibody is, as a rule, demonstrable within seven to ten days after the thera-

[1] Weil, R., *Proc. Soc. for Exp. Biol. and Med.*, 1914, xii, 37.

peutic injection, and in increasing amounts thereafter, for at least two to three weeks. As a rule, antibody can be demonstrated either at the onset of the serum sickness or within one or two days thereafter. Thus, these two factors coexist in the blood throughout the serum sickness, and even for some days after its subsidence.

The bearing of these facts upon the interpretation of serum sickness is not easy to determine. In the first place, it is not probable that the coëxisting antigen and antibody correspond to one another; in all likelihood they represent different fractions of the complex antigen, namely horse serum. According to present conceptions, the antigen is progressively neutralized by the antibody, both of the cells and of the circulating blood. Reasoning by analogy, this neutralization, when it takes place within the blood, produces no symptoms of any kind. When it takes place within the cells, it produces a multiform group of symptoms, summarized under the name of anaphylaxis. It is probable, therefore, that the serum sickness represents the progressive combination of cellular antibody with antigen. Why the symptoms should cease while the antigen is still in the blood, is perhaps explainable upon the theory that the desensitized cells failed to react further.

39 (1217)

Some suggestions for rational auto-serum therapy.

By J. BRONFENBRENNER and M. J. SCHLESINGER.

[*From the Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Pa.*]

Assuming that certain dermatoses as well as intestinal and respiratory disturbances may be due to hypersensitiveness of certain individuals to various proteins, a number of investigators suggested that the onset of acute pathologic phenomena in such cases may be due to the appearance in the circulation of specific protein, causing anaphylactic reaction. On the basis of this assumption various authors have successfully applied the method of immunization in the treatment of such conditions. In cases

where the identity of protein in question was obvious from the history of the case, or where the different methods have permitted to disclose its nature by a special investigation, the treatment consists in immunization of patient by repeated injections of the specific protein. In those cases where the nature of protein could not be determined, it was suggested to use the blood of the patient, as a carrier of antigen. If the presence of antigen in the blood of a sensitized individual causes anaphylactic phenomena, it is evident that during the periods of freedom from symptoms the patient's blood is probably free from circulating antigen. This fact is lost sight of by many authors who in applying the auto-serum therapy presumably on the basis of the above described theoretical considerations collect the blood at regular intervals without any regard as to whether such blood contains free circulating antigen or not.

Much has been written of late on therapy by parenteral introduction of nonspecific proteins[1] and it is possible, if the observations of the clinicians are correct, that the therapeutic effects of promiscuous injections of patient's own serum are due to some other phenomena than that of immunization by the circulating antigen.[2] Though in such cases apparently injections of normal horse serum can be expected to give just as good results, human serum is evidently to be preferred in order to avoid an additional sensitization to a horse protein. In so much, however, as the authors base their therapy on hypothesis that serum contains the circulating antigen, it seems essential to withdraw the blood at the time when antigen is present in it.

On the other hand, should the removed portion of the blood contain the circulating antigen, what is the rationale of injecting it back practically immediately (as some authors do), when the remaining blood of the patient contains at the time considerably larger amount of antigen already. On the basis of our experimental data we wish to suggest that the blood be withdrawn immediately preceding, during, or immediately after the anaphylactic reaction;

[1] Miller and Lusk; Smith; Jobling and Petersen. *Journ. A. M. A.*, 1916, p. 1753–58.

[2] M. H. Kahn and H. W. Emsheimer, *Arch. of Internal Medicine*, Vol. xviii,, pp. 445.

that the presence of circulating antigen in the blood be determined either by titration of its complement[1] or by antitryptic index,[2] and finally that the serum containing the circulating antigen be properly preserved and injected into patients between the anaphylactic attacks. The time of injections will be determined by antitryptic index, as the animal experiments have shown that such injections give best results at the time when antitryptic index is lowest.

Abstracts of the Communications, Pacific Coast Branch.

Fifteenth meeting.

San Francisco, California, December 6, 1916.

40 (1218)

Note on a toxic nucleoprotein obtained from rat carcinoma.

By Theodore C. Burnett.

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

Flexner-Jobling rat carcinomata of the strain used by Robertson and the author[3] were ground in a mortar with sand, extracted with M/6 NaCl solution, filtered and centrifuged to remove all foreign particles. The supernatant fluid was poured off, diluted with ten times its volume of NaCl solution, and CO_2 allowed to bubble through it for half an hour. A flocculent precipitate which settled in a few hours was the result. Phenol was added to make the suspension 0.5 per cent.

The original problem was to ascertain if such a substance, assumed to be cell globulin, would prove to be specific, using complement fixation as a test. An attempt was made to immunize a rabbit by intravenous injection, but the rabbit died within five

[1] Bronfenbrenner and Schlesinger, in press, these *Proceedings*.

[2] Bronfenbrenner, Proc. Soc. Exper. Biol. and Med., 1915, xiii., p. 42.

[3] Robertson and Burnett, *Jour. Exper. Med.*, Vol. 21, 1915, p. 281.

minutes after the injection of 3 c.c. (about 50 mgm. dried substance) on the third day. In another rabbit 4 c.c. intravenously proved fatal in ten minutes. In another, 2 c.c. on the first day were without effect, but the following day 2 c.c. caused death promptly. The symptoms are convulsions and cessation of respiration before the heart beat. Immediate post mortem reveals nothing distinctive. In one case there were punctate hemorrhages in the thymus. Others have been negative. There is no intra-vascular clotting, but on the contrary the blood from the heart remains fluid for over an hour.

Intraperitoneal injections do not result fatally, but seems to affect the animal seriously with loss of weight and a generally poor condition of nourishment. Not enough work has been done, however, to be sure on this point. At any time during the intraperitoneal injections, an injection intravenously will promptly cause death. One attempt at immunization proved negative to complement fixation.

What has been said of rabbits is also true for white rats, excepting that intra-peritoneal injections make them sick for an hour or so, after which they recover.

As to the substance itself, it is tentatively assumed to be a nucleoprotein. It is weakly positive to the Biuret test, but the color develops slowly. It is negative to the xanthoproteic, positive to the Adamkiewicz and Millon's. It gives a positive test for pentoses with orcin. It is practically insoluble in water and 5 per cent. NaCl, but dissolves readily in tenth normal NaOH. The presence of a pentose places it in the group of nucleoproteins and as it is toxic and probably non-antigenic it behaves like a nucleohiston. Many questions at once suggest themselves and they will be discussed in a later communication. Meanwhile work on the original problem is being carried on.

41 (1219)

The absorption of phenolsulphonephthalein from the subarachnoid space in diseases of the central nervous system.

By HENRY G. MEHRTENS, M.D. and HOWARD F. WEST, M.D. (by invitation).

[*From the Division of Medicine, Stanford University Medical School, San Francisco, Cal.*]

Dandy and Blackfan have shown that the injection of phenolsulphonephthalein into the subarachnoid space in man is harmless and in normal individuals appears in the urine in from six to ten minutes. They also found in certain cases of hydrocephalus that there is a delay in the excretion of the dye. So far as we know no determinations have been made of this delay in other pathological conditions.

A series of sixty-one patients, mostly with nervous diseases, received injections of phenolsulphonephthalein after lumbar puncture and the appearance time of the dye in the urine was observed. These cases may be divided roughly into four groups, as follows:

I. Thirteen cases in which there was neither physical nor spinal fluid evidence of central nervous disease, nor symptoms suggesting organic nervous lesions. Appearance time was from four to fourteen minutes—eleven appeared in ten minutes or under—averaging nine minutes.

II. Five cases with neither physical nor laboratory findings positive, but with definite symptoms suggesting organic nervous disease, as follows: Two patients having positive blood Wassermanns and symptoms of paresthesias, pain and mental disturbance, one patient with aphasia, one with pains, nervousness, and loss of sexual power, and one with dizziness and paresthesia. Appearance time was from thirteen to forty minutes, averaging thirty minutes.

III. Twelve cases having positive physical findings, but with negative spinal fluids except for an occasional increase in pressure. Appearance time fifteen to seventy minutes, averaging thirty-nine minutes.

IV. Thirty-one cases having both physical and laboratory evidence of central nervous system disease. Appearance time fourteen to eighty minutes, averaging forty-six minutes.

In a general way, delay in excretion ran parallel to the severity of the clinical symptoms, but the quantity eliminated has appeared to be more variable. The method may prove of value in detecting instances of organic central nervous system disease in which the ordinary spinal fluid findings are negative, and since the absorption from the subarachnoid space is believed to be through the blood vessels the delays observed may serve to indicate the degree of vascular change.

42 (1220)

Analysis of the anaphylactic reaction by means of the isolated anaphylactic lung.

By W. H. MANWARING and YOSHIO KUSAMA.

[*From the Department of Bacteriology and Immunology, Leland Stanford Jr. University.*]

Analyses of the anaphylactic and immune reactions by means of perfusion experiments with the isolated guinea-pig lung show that we are here concerned with three essential physiological factors:

(*a*) *Cellular Anaphylaxis* or the anaphylactic response of the hypersensitive fixed pulmonary tissues,

(*b*) *Humoral Anaphylaxis*, or the chemical reactions (anaphylotoxin formation) with the anaphylactic blood elements.

(*c*) *Humoral Immunity*, or the inhibiting and protective action of the immune blood elements.

In the 14-day anaphylactic guinea-pig, the fatal bronchial spasm is due in part to cellular hypersensitiveness, in part to humoral anaphylaxis.

In the 4-week anaphylactic guinea-pig, the humoral reaction is always very slight and usually absent. The cellular hypersensitiveness, however, is usually greater than that of the 14-day anaphylactic guinea-pig. The fatal bronchial spasm in the 4-week anaphylactic guinea pig is usually due solely to the cellular hypersensitiveness.

In the immune guinea-pig, or guinea-pig that has received multiple injections of the foreign protein, the cellular hypersensitiveness is usually greater than that of the 4-week anaphylactic guinea-pig. A bronchial spasm, however, is prevented by the inhibiting and protective action of the immune blood elements.

In the immune guinea-pig, therefore, we have a paradoxical phenomenon, the coexistence of a cellular anaphylaxis and a humoral immunity. A removal or reduction of the humoral immunity leads to a fatal bronchial spasm in the immune guinea-pig.

SCIENTIFIC PROCEEDINGS.

Abstracts of Communications.

Eightieth Meeting.

43 (1221)

College of Physicians and Surgeons. President Jacques Loeb in the chair.

Changes in the electrocardiogram due possibly to alterations in blood volume.

By **R. A. Morison, M.D.** (by invitation.)

[*From the Hospital of the Rockefeller Institute for Medical Research, New York.*]

There are reported in this communication certain changes fo the electrocardiogram, which are supposed to have been influenced by changes of the blood volume in patients. The changes are in the latter part of the ventricular complex, the T wave, and appear in one patient in the most marked extent in the first lead and in another patient most markedly in the third lead.

The first patient suffered from chronic hypertensive nephritis, cardiac hypertrophy and arterial disease. Before bleeding, an electrocardiogram was made, using the second lead only. He was bled 500 c.c. Afterward, with the same resistance, the same tension of the string, the same strength of magnetic field, and with the same position of the patient, a second electrocardiogram was made and showed a much increased amplitude of the T wave in the second lead. Leads one and three were not taken. This patient was then given a test in which he drank 1,500 c.c. of water in fifteen minutes. His electrocardiogram was made before the beginning of the test and about every hour thereafter for ten hours; then at varying periods until about twenty-three hours had elapsed. Six hours and thirty-seven minutes after he had

finished drinking the water, the T wave which had been diphasic in the first lead, became positive and remained positive for twelve hours and eighteen minutes. During this time the patient had not ingested any food or fluid. Twenty hours and fifteen minutes after ingesting the water, he was given 300 c.c. of fluid. The form of the electrocardiogram made immediately after this showed its form to be the same as that of the control.

The second patient is one suffering from hypertrophy of the heart and arterial disease. He was given the same test as the preceding patient. The control electrocardiogram showed in the third lead a diphasic T wave, the positive part of it being of greater amplitude than the negative part. Five minutes after the patient had finished drinking the water (twenty minutes after the beginning of the test), the T wave had become wholly negative. The T wave remained negative for sixteen hours and thirty-five minutes, when it became diphasic. This change occurred without the patient having had additional fluid. On another occasion, this patient was given two hot packs and showed the same variation in the third lead. He lost 0.95 kg. He then drank 200 c.c. of milk. The electrocardiogram now resumed the form of the control.

This test has been made on a normal man, in whom no qualitative change occurred.

In the above tests the resistance of the patients varied from 800 to 1,300 ohms, except once when the resistance was 2,000 for each of three leads and on two other occasions when it lay between 1,600 and 1,700 in each of three leads.

Conclusion: Bleeding, hot packs, ingestion of water and fasting may in certain persons affect the form of the electrocardiogram.

44 (1222)

The influence of ergotoxin on the pupil of the rabbit.

By T. S. GITHENS.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

In the very careful studies of Dale on the pharmacodynamic action of ergotoxin, it is stated, without qualification, that this

drug causes a contraction of the pupil. His studies were made on cats.

I have found that in rabbits, ergotoxin produces exactly the opposite effect. That is to say with all doses a dilatation of the pupil lasting several hours results. With doses of 1 mg. per kilo, which did not cause any marked general intoxication, the pupil dilated to 7½ mm. from an original size of 5 mm. With doses of 2 mg. per kilo, the pupil reached a size of 9 to 10 mm. All injections were in the ear vein.

These larger doses cause a certain amount of disturbance of the respiration, and it might be claimed that the dilatation was due to asphyxia. In fact Dale ascribes a dilatation mentioned by Kobert in a protocol, to this cause. In order to exclude this factor I curarized a number of rabbits and under artificial respiration, injected ergotoxin. The heart was not markedly disturbed, but the dilatation of the pupil was even greater than in most of the normal animals.

We may then assume that the dilatation seen in the rabbit's pupil is not secondary to asphyxia but is due to the action of the drug itself.

Dale states that ergotoxin exerts two distinct actions on the sympathetic nervous system. First a stimulation of the muscle fibers of certain organs, notably the uterus. Second a paralysis of the motor myoneural junctions of the true sympathetic. It is to the latter action that the constriction of the cat's pupil is said to be due. It is possible either that ergotoxin acts in a different manner in the two species, or that the first action, direct stimulation of the muscle fibers is exerted on the dilator muscle of the rabbit's pupil and overcomes the effect of paralysis of the myoneural junction. The view that ergotoxin acts at least in part, directly on the muscle fiber, is perhaps favored by the occurrence of dilatation from ergotoxin, in two rabbits which were anesthetized with ether until the light reflex was abolished.

That the action of ergotoxin is not exerted on any more central structure of the dilating mechanism is proved by the fact that its action is little influenced by ganglionectomy.

I recently reported that in rabbits, ergotoxin caused a very marked rise of temperature. That the pupil dilatation is not

associated with this, is shown by the fact that the dilatation reaches its maximum within 15 minutes, at a time before the temperature has begun to rise. It is of interest to note, however, that the two seemed almost always to parallel one another. The wider the dilatation of the pupil, the higher was the subsequent fever.

45 (1223)

The excretion of Congo red by the stomach.

By **R. L. CECIL** and **R. WEIL.**

[*From the Department of Experimental Medicine, Cornell University Medical School, N. Y.*]

The excretion of dyes by the stomach has not as yet been studied in human beings, and even in animals has been investigated only to a very slight extent. Abel found that phthalein dyes were not excreted by the stomach in animals. For several years we have been engaged in the study of the diazo dyes, one of us[1] having paid particular attention to the effects of Congo red when injected intravenously into human beings. The present communication gives a preliminary report on the excretion of Congo red by the human stomach in conditions of health and disease. We have injected up to one gram intravenously into human beings and found that the normal stomach fails to excrete the dye, except in very small amounts, even when these maximal doses are used. As a matter of routine, we have injected 0.3 or 0.4 gm. in normal salt solution.

One of us has shown that anilin dyes, when injected intravenously, may be discovered in the secretions of external ulcers and of ulcerated cancers. With this fact in mind, it seemed advisable to examine cases of gastric ulcer and cancer after intravenous injections of Congo red. We have found in a limited series of such cases, that, as a matter of fact, the dye may generally be demonstrated in relatively considerable quantity in the stomach contents. It is not in solution, but upon filtration is deposited upon the filter paper. Microscopically, granules of the dye can be detected

[1] Weil, R., *Jour. of Cancer Research*, 1916, I, 1.

lying free or within phagocyte cells, and it seems probable that it is excreted chiefly, if not entirely, in these cells. Bleeding from the surface of the ulcers is not responsible for the presence of the dye in the stomach contents. The dye is first present in demonstrable amounts from forty-five minutes to one hour after injection, and may be found as long as three hours or more thereafter. The optimum time for withdrawal of the contents is probably about one and one half hours. The diagnostic value of the method in ulcerated conditions of the gastric mucosa is a subject which is being further studied, and which will require a very considerable series of examinations to determine finally. From the limited number of cases yet examined, it is impossible to draw any conclusions further than that in the pathological conditions above mentioned the dye is frequently present in considerable amount in the contents. It is possible that other conditions, such as chronic gastritis or congestion, may permit of the excretion of the dye in a similar manner and in comparable amounts, but we have not as yet found this to be the case. As regards the excretion of the dye in the duodenum, we are not in a position to make any report. The fact that the dye is normally excreted in the bile in solution presents certain difficulties in the study of this problem.

46·(1224)

A comparative test of different antigens and of different temperatures of incubation in the Wassermann test.

By J. WHEELER SMITH, JR., and W. J. MACNEAL.

[*From the Laboratories of the New York Post-Graduate Medical School and Hospital.*]

Tests were performed by six different methods upon 500 identical specimens from 457 patients. Three antigens were employed, cholesterinized alcoholic extract of beef heart, simple alcoholic extract of beef heart and the acetone-insoluble lipoid fraction of alcoholic extract of beef heart, prepared according to the method of Noguchi. Each of these antigens was used at two different incubation temperatures for fixation of the complement,

37° C. and 8° C., the subsequent incubation after addition of sensitized erythrocytes being carried out at the higher temperature.

Upon known syphilitics, the cholesterinized antigen at 8° C. gave the largest number of positive reactions, being followed, in order of efficiency, by the plain antigen at 8° C., cholesterinized antigen at 37° C., acetone-insoluble antigen at 8° C., the same at 37° C., and last the plain antigen at 37° C.

Reactions considered to be false positives were obtained eight times with the cholesterinized antigen at 37° C., five times with the cholesterinized antigen at 8° C., and once with the plain antigen at 8° C., in this series of 500 tests.

47 (1225)

On thyroidectomy in amphibia.

By **E. R. HOSKINS** and **MARGARET MORRIS.** (*By invitation.*)

[*From the Department of Anatomy, N. Y. University and Bellevue Hospital Medical College and Department of Zoölogy, Yale University.*]

With due care to technique it was possible to remove successfully the anlage of the thyroid gland from young growing larvæ of *R. sylvatica* and *Amblystoma punctatum.* The stage best suited for this experiment is that just preceding the beginning of the circulation of the blood. At this time there is no danger of hemorrhage and the chances of regeneration of the removed gland are fewer than with younger larvæ. Chlorotone in salt solution was used to produce anesthesia.

Thyroidectomy was performed in 40 frog larvæ and 50 *Amblystoma* larvæ checked against an equal number of control animals.

A few of the thyroidectomized frog larvæ developed abnormally shaped external gills in some of which no circulation was to be seen. This was evidently due to injury to the vascular system. One animal developed no external gills although it lived and grew through the period during which external gills normally persist.

The operated animals grew less rapidly than the controls. Only one control and one experimental animal survived the normal

period of metamorphosis. Of these the control showed hind legs two months after the operation and the other had not developed legs four months after the operation.

Serial sections were made of eight experimental frog larvæ. The operation was seen to have prevented development of the thyroid gland in all but one case. The hypophysis as compared with that of the controls showed no changes in size or structure to be attributed to loss of the thyroid gland.

Among the *Amblystoma* larvæ none developed abnormal gills. The average growth rate of the experimental larvæ was less than that of the controls, but of the fourteen which were alive, after three months, the largest had had the thyroid removed. In none of the thirteen operated animals that were sectioned was there any regeneration of the thyroid. There were no changes in the hypophysis nor in the pigmentation of the skin following the thyroidectomy during the three months in which the operated larvæ were under observation.

48 (1226)

The effect of decerebration upon the quick component of labyrinthine nystagmus.

By F. H. PIKE.

[*From the Department of Physiology of Columbia University.*]

The effects of decerebration have been variously held to show (1) that the central cells of certain reflex mechanisms are located in certain definite regions or levels of the central nervous system. and (2) that certain other reflex mechanisms do not have their central representation in the same regions or in other regions, There has been little consistency in drawing conclusions from the results of decerebration, and frequently other considerations have entered into the matter to such an extent as to outweight the experimental results of decerebration. In addition, the experimental results as reported by various investigators are not in agreement, and one notices a lack of post-mortem reports as to the extent to which destructive hemorrhages have burrowed downward from the level of transection of the brain stem, or on other conditions

which may affect the interpretation of the results. And the procedure itself is supposed to produce shock through its great trauma or not to produce shock, according to the demands of the hypothesis which is to be sustained. Sherrington's[1] statement that trauma qua trauma has little or nothing to do with the onset of shock is frequently overlooked.

The various reflex mechanisms associated with the eye have been investigated largely in decerebrated animals. Mayo[2] decerebrated a pigeon leaving the optic tubercles and the crura cerebri, and then cut below the medulla oblongata. The optic and the third nerves were left intact within the cranium. On pinching the central end of the stump of the optic nerve of one side, the iris contracted. Mayo states that contraction of the pupil could be obtained immediately after decapitation—a violent surgical procedure. The conjunctival reflex in the rabbit seems to involve the myelencephalon as well as the metencephalon.[3] It persists unaffected by shock, when transection is made in front of the pons.

The slow deviation of the eyes in response to stimulation of the otic labyrinth persists after decerebration and after splitting the entire metencephalon in the mid line, but the quick component of nystagmus—the quick jerk of the eyes back to the mid line or to the line of vision—is abolished. Removal of one cerebral hemisphere abolishes the quick movement when the slow movement of the eyes is directed to that side, but does not affect the quick movement when the slow movement of the eyes is directed to the side of the remaining cerebral hemisphere.

In view of the facts that (1) decerebration does not affect the magnitude of the slow movement of the eyes in response to labyrinthine stimulation, (2) removal of one cerebral hemisphere affects the quick movement of the eyes in one direction only, (3) that the quick movement of the eyes is abolished when only the temporal and basal portion of the cerebral hemisphere of one side is re-

[1] Sherrington, "Integrative Action of the Nervous System," New York, 1906, pp. 241–243.

[2] Herbert Mayo, "Anatomical and Physiological Commentaries," London, 1823, Vol. II. pp. 4, 18, 136; cited by Sherrington, Schäfer's "Text Book of Physiology," 1900, Vol. II, p. 812.

[3] Exner, *Archiv für die gesammte Physiologie*, 1874, VIII, p. 530; Schäfer's Text Book of Physiology, 1900, II, p. 892.

moved just as when ablation of the whole hemisphere is made, (4) the natural history of nystagmus, (5) the development of nystagmus in young animals[1] and (6) the known cerebral localization of the motor cells, stimulation of which produces ocular movements, it is probable that the quick component of nystagmus is a reflex response, a part of whose path lies through the cerebral hemispheres. The reasons for invoking shock are not clear.

It is probable also that the quick component of labyrinthine nystagmus has no necessary connection with the labyrinth, but that it is a reflex[2] whose afferent impulses arise from stimulation of the afferent endings in the eye muscles.[3] The cortical end stations of fibers from these afferent endings are, as we now believe, in the temporal region. The quick component of nystagmus has developed along with the greater degree of mobility of the eyes, and brings about the return of the eyes to a position such that the original line of vision is restored when they are deflected too far to one side.

49 (1227)

The influence of certain conditions on the rate at which epinephrin is liberated from the adrenals into the blood.

By G. N. STEWART and J. M. ROGOFF.

[*From the H. K. Cushing Laboratory of Experimental Medicine of Western Reserve University, Cleveland, Ohio.*]

1. By means of the rabbit intestine and uterus segment tests, we have obtained further evidence that, under our experimental conditions at any rate, the rate of discharge of epinephrin into the blood of the adrenal veins is relatively steady and not easily influenced by such procedures as we have tried; for example, stimulation of the afferent fibers in large peripheral nerves (sciatic and brachial) or asphyxia. This is not because the discharge is already maximal owing to the necessary conditions of the experi-

[1] Prince, *American Journal of Physiology*, 1917, XIII, p. 308.

[2] Wilson and Pike, International Congress of Medicine, London, 1913, Section XLV, p. 563.

[3] Tozer and Sherrington, Proceedings Royal Society, London, B, LXXXII, 1910, p. 450.

ment (trauma, anesthesia, etc.). For, by electrical stimulation of the cut splanchnic, the rate of liberation can be made decidedly greater than the rate of spontaneous liberation with intact splanchnics.

2. Unlike the rate of liberation per unit of time, the concentration of epinephrin in the adrenal vein blood can be observed to vary decidedly in the course of an experiment, increasing, in general, as the rate of blood flow decreases. This can be shown by collecting adrenal vein blood in successive samples. When the blood flow slackens, owing to hemorrhage or other circumstances, the earlier specimens will be found to contain a smaller concentration of epinephrin than the later specimens.

For example, in a dog, weighing 10 kg., the first sample from the cava pocket, into which the adrenal veins were alone discharging, flowed at the rate of 8 c.c. per minute, the second sample 7.2 c.c., third 5.8 c.c., fourth 4.4 c.c., fifth 3.2 c.c., sixth 2.4 c.c., seventh 1.5 c.c. A definite increase in the epinephrin concentration in the successive samples was clearly shown by the intestine and especially by the uterus tests. The concentration was assayed in the first sample at somewhat more than 1 : 3,300,000; in the third sample at somewhat more than 1 : 1,670,000; in the seventh sample at somewhat less than 1 : 750,000.

The increase in the concentration in the blood is far too great to be accounted for by any increase in the relative proportion of plasma to corpuscles associated with hemorrhage without change in the concentration of epinephrin in the plasma. And it has been demonstrated that the sera separated from the successive samples of blood show a progressively increasing concentration of epinephrin.

Even when the circulation through the adrenals is stopped altogether by clamping the veins, the liberation of epinephrin into the pent-up blood continues for a time at an apparently undiminished rate and the concentration of epinephrin in the blood must go on increasing.

3. For the reason mentioned in paragraph 2 it is not in general permissible to deduce changes in the rate of liberation of epinephrin from changes in its concentration in the adrenal vein blood, unless the rate of blood flow through the adrenals is known. Changes in the concentration of epinephrin in the blood of the inferior cava above the adrenals can be produced by alterations in the rate of blood flow in the cava, even where the rate of liberation of epinephrin from the adrenals has remained constant.

4. No evidence has been obtained that after section of the nerves of one adrenal, any compensatory increase in the rate of liberation of epinephrin from the other gland occurs. The fact that section of one splanchnic diminishes the discharge of epinephrin by a half, without causing any material fall of blood pressure, affords additional evidence that the epinephrin discharged by the adrenal veins is not directly a factor, or at least not an important one in maintaining the blood pressure.

50 (1228)

The proportion in which adrenalin distributes itself between corpuscles and serum in relation to the technique of testing for epinephrin in blood.

By G. N. STEWART and J. M. ROGOFF.

[*From the H. K. Cushing Laboratory of Experimental Medicine of Western Reserve University, Cleveland, Ohio.*]

1. When adrenalin was added to defibrinated blood, and the blood centrifuged after an hour, the serum was found by the colorimetric method of Folin, Cannon and Denis, to contain practically the whole of the added adrenalin.

3 c.c. adrenalin solution (Parke, Davis & Co.), corresponding to 2.64 mg. epinephrin when assayed colorimetrically, was added to 30 c.c. cat's defibrinated blood. Correcting for the small amount of color given by the serum itself in the test, the amount of adrenalin found in 10 c.c. of the serum separated from the adrenalin blood corresponded to 1.37 mg. epinephrin. The proportion by volume of serum in the blood was 62 per cent. The amount of serum in 30 c.c. of the adrenalin blood would, therefore contain $1.37 \times 30 \times 62/100 = 2.55$ mg. adrenalin, *i. e.*, all the adrenalin added was in the serum.

2. The same result was obtained by assaying the adrenalin in the serum by injection into a pithed cat (method of Elliott). The serum gave a rise indicating, when compared with that given by a known amount of adrenalin in control serum, that 10 c.c. of it contained 1.32 mg. adrenalin. This compares with 1.37 mg. by the colorimetric method. The adrenalin blood gave a rise of blood pressure less than that given by the serum and corresponding to the concentration of adrenalin in it. The sediment, which of

course contained a very small proportion of serum, gave no measurable rise.

3. Similar results were obtained (with dog's blood to which adrenalin had been added) on segments of rabbit's intestine and uterus. The sediment gave a small inhibition of the intestine and a small increase of tone of the uterus as compared with the serum. The effect of the adrenalin blood was intermediate in amount.

4. The distribution of the naturally secreted epinephrin in the blood from the adrenal veins (of the dog) was also investigated with the same result. Only the rabbit intestine and uterus were employed, the other methods not being sufficiently sensitive for the small concentrations found in blood. In one experiment the concentration of epinephrin in the blood was assayed at 1 : 8,000,000, in the serum at 1 : 3,000,000. The sediment gave practically nothing. It so happened that the blood used was extremely rich in corpuscles, a circumstance favorable rather than otherwise for testing the point in question, as the serum would be more than ordinarily rich in epinephrin as compared with the blood, if all the epinephrin is contained in the plasma. The proportion of serum by volume in the blood was 36 per cent. On the hypothesis that all the epinephrin was in the serum, this would give 1 : 100/36 × 3,000,000, *i. e.*, 1 : 8,300,000 as the concentration in the blood.

5. When search is being made for the minute quantities of epinephrin present in blood, serum (or plasma) should, in general, be preferred to blood in making the tests.

51 (1229)

The influence of intravenous inoculations of cholesterin upon blood cells.

By OSKAR KLOTZ and MARY W. SPENCER.

[*From the Pathological Laboratories, University of Pittsburgh, Pittsburgh, Pa.*]

Some years ago (1907) Talquist believed that he had found the harmful substance present in the *Bothriocephalus latus* leading to progressive anemia. The substance which he isolated from these

worms was a cholesterinester present in greatest proportion as cholesterin oleate. He found that a synthetic cholesterin oleate did not have such active hemolyzing properties as the extracts from the worm, but on his general findings he believed that this substance was the cause of the anemia and suggested the possibility of other anemias arising through the action of similar substances. Since this time cholesterin in various forms has been used to a considerable extent for other experimental purposes. In some of these experiments the materials were fed to animals while in others they were introduced by inoculation. Depending upon the dosage there was a variable increase in the cholesterin content of the blood. This cholesterin was present in combination with fats or lipoids. Even with the development of a continued hypercholesterinemia amounting to several times the normal blood content, none of the authors have remarked upon the production of a progressive anemia. In our own feeding experiments no anemia was apparent, although the cholesterin of the blood was often very high.

Recently we have studied the effect of the direct introduction of cholesterin combinations into the blood. An emulsion of a cholesterin combination with sodium oleate, containing 7.5 per cent. of cholesterin and 5 per cent. of sodium oleate, was used. The cholesterin in these materials forms a combination with sodium oleate so that colloid globules remain in suspension and are readily introduced into the circulation of animals. The cholesterin in this form does not give rise to a foreign body reaction as when the pure cholesterin is used. Furthermore, this mixture does not show the active hemolysis in the test tube, as is demonstrated by the same quantities of sodium oleate.

Two rabbits were treated every second day by intravenous inoculation of 2 c.c. of the emulsion for a period of two weeks, while a third received from ½ to 1 c.c. during a similar period. Counts were made prior to the initial inoculation to determine the normal for each animal. Counts were also continued for ten days after the last treatment. In none of the animals were we able to observe any effect of the inoculated material upon the red blood cells. In the normal rabbit we have found a fluctuation between six and seven and a half million red cells and at no time in the ex-

periments was there any appreciable decrease below the normal minimum. There was no alteration in the morphology or staining qualities of the red cells. Furthermore, it was found that but slight reactions occurred in the white cells of the blood. Immediately following the inoculation there was a temporary rise in the number of white cells amounting in its greatest extent to 2,000 cells above the normal maximum (10,000). This increase remained only for twenty-four hours and then the count declined to normal. The increase was not confined to any particular type of cell, though the response in the polymorphonuclear neutrophiles was more common. The experiments indicate that for the amount of the cholesterin mixture used intravenously, there is no particular reaction in the blood cells of this animal. There was no evidence that the cholesterin macrophages appearing in organ lesions during hypercholesterinemia, migrate by the blood stream.

52 (1230)

The physical state of antigen as related to the specificity of the Wassermann reaction.

(Preliminary Communication.)

By J. BRONFENBRENNER and M. J. SCHLESINGER.

[*From the Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Penna.*]

The shortcomings in the diagnostic value of the Wassermann reaction have been demonstrated by many authors. Of late, however, in addition to errors inherent to this reaction on account of its very nature, different investigators called attention to discrepancies arising from the use of various modifications. There is a definite tendency among the serologists to standardize the Wassermann test as a whole and thus make the results obtained by different workers comparable. In view of facilitating this standardization, we wish to call attention to certain qualities of antigen which have not been described thus far.

So far as the chemical composition of antigen is concerned, the

pure lipoid (acetone insoluble fraction of tissue lipoids) properly prepared is, in our experience, by far superior to any watery or alcoholic extracts, as well as to those reinforced by cholesterin.[1] We found, however, that in order to obtain constant results it is not sufficient to merely ascertain the chemical composition of antigen, but its physical state as well. By changing the method of emulsifying the alcoholic solution of acetone insoluble tissue lipoids in salt solution, we obtained emulsions which were essentially different from one another. In general, the emulsions can be divided into two groups: those opaque and those only slightly opalescent and fluorescent. The two types are essentially different; thus no amount of dilution of the opaque emulsions will give them the fluorescent appearance characteristic for the second group of emulsions even though the degree of opalescence can be approached.

We found that the results of Wassermann tests performed on the same sera with these two emulsions give different results, and in general the opaque emulsions are more anticomplementary and the fluorescent are more antigenic. The opaque emulsions of antigen give the results comparable to those obtained with cholesterinized alcoholic extracts, namely, one obtains more positive reactions in treated cases as well as in a certain percentage of normal cases and misses a number of reactions in early stages of syphilis, whereas the fluorescent-opalescent emulsions, though missing a certain number of treated cases, are very much more sensitive and specific at the same time.

53 (1231)

The influence of subcutaneous injections of morphine upon the hydrogen ion concentration of the urine in the dog and rabbit.

By FRANK P. UNDERHILL, NORMAN L. BLATHERWICK and SAMUEL GOLDSCHMIDT.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.*]

The subcutaneous administration of morphine (morphine sulphate, 10 mg. per kilo body weight) to fasting dogs results in the

[1] We are reporting elsewhere a comparative study with different antigens on over 20,000 cases.

excretion of a strongly alkaline urine, which may persist for a period of twenty-four hours or more. The alkalinity is undoubtedly due to the presence of carbonates since addition of acid to the urine causes effervescence of carbon dioxide. After the urine has resumed its usual acid character a subsequent injection of morphine may fail to elicit an alkaline urine although the hydrogen ion concentration may be diminished appreciably. On the day of morphine introduction there is usually a definite increase in the elimination of the total urinary nitrogen.

Rabbits fasted until they excrete a strongly acid urine show no significant change in the hydrogen ion concentration of the urine nor is the total nitrogen altered even though very large doses (80 mg. per kilo) of morphine are subcutaneously introduced. These results are in accord with the well-known resistance of rabbits to morphine narcosis.

54 (1232)

The determination of oxygen in blood.

By DONALD D. VAN SLYKE.

[*Hospital of the Rockefeller Institute for Medical Research, N. Y.*]

This requires 5 minutes for human blood, unless a little sapanin has been added to the ammonia, in which case 15 seconds may suffice for the laking.

The apparatus for determining carbon dioxid in blood, described in the Proceedings for the meeting April 21, 1915, can be used with equal facility and accuracy for determination of oxygen. 6 c.c. of ammonia made by diluting the concentrated solution with 200 parts of water, are introduced into the apparatus with 5 drops of caprylic alcohol. The apparatus is evacuated and the air extracted from the ammonia by shaking for a few seconds. The extracted air is expelled and the process completed to make certain that none is left.

2 c.c. of blood are then introduced. The blood and ammonia are mixed and allowed to stand until the blood is laked. Then half a c.c. of saturated potassium ferricyanide solution is in-

troduced (the cyanide solution is made air-free by boiling or by shaking in an evacuated flask, and is kept in a burrette under a layer of paraffin oil two or three centimeters thick to exclude air). The apparatus is now evacuated, shaken and the oxygen set free determined exactly as is carbon dioxid. The solubility of oxygen in water is so slight that no correction is made for what remains in solution. The only correction necessary is for the small amount of nitrogen gas which 2 c.c. of blood contain.

SCIENTIFIC PROCEEDINGS

Abstracts of Communications.

Eighty-first meeting.

College of the City of New York, February 21, 1917.

President Jacques Loeb in the chair.

55 (1233)

Pervaporation, perstillation and percrystallization.

By Philip Adolph Kober.

[*From the Division of Laboratories and Research, N. Y. State Department of Health, Albany, N. Y.*]

In the course of some experiments on dialyzation, my assistant, Mr. C. W. Eberlein, called my attention to the fact that liquid in a colloidion bag, which was suspended in the air, evaporated, although the bag was tightly closed. At first we were inclined to ascribe it to evaporation through a small aperture at the top of the bag, but further experiments and especially the speed of evaporation soon forced us to the conclusion that the aqueous vapor is given off through the membrane, as though the water were suspended as a solid without any membrane present. This phenomenon we have named pervaporation. The speed of this pervaporation is so great that with ordinary heating facilities such as a Bunsen flame and electric heaters, it has been impossible to heat water in a collodion container to a boil.

Distillation by means of pervaporation we have called perstillation. When a dialyzable solute within the membrane container reaches saturation, it crystallizes on the outside of the membrane. This phenomenon we have named percrystallization. In order to show some of the possible uses of these phenomena, we described a number of experiments and discussed the theoretical considerations.

For details see April number, Journal American Chemical Society, 1917.

56 (1234)

A contribution to the metamorphosis of skin in amphibians.

By EDWARD UHLENHUTH.

[*From the Laboratories of the Rockefeller Institute for Medical Research,*]

Gudernatsch has shown that thyroid is able to induce metamorphosis in frog and toad larvæ, if they are fed on this gland. The effect was so striking that metamorphosis sometimes started five days after thyroid was fed.

From these experiments it is however not certain that thyroid is the substance which under normal conditions causes metamorphosis, nor has it been proven by these experiments that an agent similar to its physiological character is involved in normal Amphibian metamorphosis.

In a series of experiments it has been shown, that also under normal conditions some agent must be furnished to the organs of the animal in order to bring about metamorphosis of these organs. It has turned out that the organs themselves are unable to produce this agent and that it is the body which produces the agent, *i. e.*, some place or organ in the animal's body remote from the organs examined. If this agent is prevented from reaching the organ, it will not metamorphose.

The general method of these experiments is to remove the organs from the animal's body, before the body begins to produce the agent and to graft it to another animal in which the agent will not be produced for a long time. By means of this metamorphosis of the organ can be inhibited for as long as the new host does not metamorphose; in this way metamorphosis could in some cases be delayed by seven months. Until now the eyes, the skin and the gills—the latter by Kornfeld—were studied in this way. Today we will report mainly on experiments performed on the skin of *Amblystoma punctatum.*

In order to make you familiar with the changes occurring in the skin of this animal during development I will show a few pictures.

The first one (Fig. 1) shows a normally fed animal, still being larval; the skin is then reddish or yellowish brown without any particular patterns (*N* 15). The next stage is brought about by the development of a greenish or yellowish network on the brown background. Color and definiteness of this network depend upon the kind of food and on the amount of light. Animals kept in bright light and fed on thymus show the greatest definiteness of this stage (Fig. 2). When the skin develops the network, the animals are still larvæ no matter how they have been treated, though rare exceptions do occur. The next stage is the separation of this network into single green or yellow spots. Fig. 3, p. 2, shows this stage in an animal which was kept in daylight and fed on thymus; such animals always reach this stage while they are still larvæ. But animals fed on worms or kept in darkness usually do not work out their first spots before they have left the water. In this case another characteristic which appears independently from the stage of separation in animals reaching this stage while they are larvæ appears simultaneously with the separation stage; namely, soon after the animals leave the water, the background changes its color to a dark reddish or sometimes greenish brown and the skin appears leathery. All colors become more dim and faint. We called this stage "Cinnamon." It is shown in Fig. 4, *N* 3. The animal then becomes darker and darker until it is finally black. The yellow spots, which have become reduced in number and size lighten up and are finally bright yellow and shining. But the number of the spots as well as the shade of the yellow are subjected to great variations which of course cannot be discussed here.

I have here a number of formalin specimens which represent different stages of the skin colorations and which may perhaps illustrate these stages better than I could explain it.

The method was then as follows:

For each experiment three larvæ of *A. punctatum* of about the same age were used. From *A* one piece of skin including one eye was grafted to *X* while the other half of the head's skin including the other eye was grafted to *Y*. 23 pairs were operated on in this way. The skin grafts in each pair were continuously compared with each other as well as with their respective hosts.

A number of animals died before results were obtained; but in about ten pairs *X* did not metamorphose when *Y* did. In this case the grafts did metamorphose simultaneously with their respective hosts but in consequence they did not metamorphose simultaneously with each other as they would have done if left with the animal *A*. As *X* and *Y* were about of the same age, the differences were mostly slight, ranging from 3 to 28 days. But in the pair 33–34, the animal 34 did not metamorphose for some reason and was still larval in January, when it was subjected to a new operation and died. In this animal the skin graft as well as the eye graft also remained in an entirely larval condition. The other piece of skin and the other eye originating from the same animal *A* as the grafts of the Exp. 34 had, on the other hand, metamorphosed already at the beginning of September, four months after which time the graft of Exp. 34 was still larval.

On October 19 both animals were photographed and painted. Fig. 5 shows animal 33; it is fully metamorphosed and black and its spots are bright and yellow. The graft is also metamorphosed and has developed three spots. In this case the graft's spots are almost orange and quite different from the host's spots, indicating particularly well their different origins. This is also interesting because it shows that the specific characteristics of the graft have not been changed by the host, though the time of metamorphosis has been so thoroughly influenced by the host. Fig. 6 shows Exp. 34, the other animal of this pair. It is still larval, having not even developed the network. The graft can be plainly seen. It is according to its different origin, slightly different in shade, but in the same color stage—of an even brown color. The eye is also larval, as it still shows the yellow ring unbroken. Both animals have been preserved in formalin. You will easily see the spots of the skin graft in animal 33; the skin graft of animal 34 has been removed from this animal and grafted to a larva of *A. opacum*, which is preserved in formalin also; in examining it you will notice the uniform brown coloration of the graft and the entire lack of any network or spots,—four months after the graft on animal 33 was metamorphosed.

Finally I would like to mention that this agent which causes metamorphosis in the skin is by no means a specific substance;

it can also be furnished to the skin by the body of another species. Fig. 7 shows a specimen of *A. tigrinum*, to which a piece of skin with the eye from a larva of *A. punctatum* was grafted. In four other specimens of *A. tigrinum* which had a similar graft, metamorphosis had occurred from 1 to 6 weeks ago; in these animals also the graft was metamorphosed. The animal shown here was still larval, when painted, as you see from its color and large gills. The graft, as you see, is also larval. The skin is even grayish brown and the eye shows the ring still. It was not until after about three weeks that this animal also metamorphosed and then, simultaneously with its host, also the graft metamorphosed. The animal was painted again on November 24, the ring of the eye is gone and only a few yellow spots, instead of the ring are left (Fig. 8).

Hence what these experiments show is that under normal conditions a certain agent induces metamorphosis in the Amphibian organs; that this agent cannot be produced by the organs themselves, but must be furnished by the animal's body; that metamorphosis can be delayed as long as the agent does not reach the organs and that this agent is a non-specific one, as it also is produced by another species.

According to the method of these experiments it was only possible to delay metamorphosis (by seven months in the case of the eye experiments on *Salamander maculosa*). I am, however, fully aware that it will be necessary to show that metamorphosis can be entirely prevented if the agent is permanently kept away from the organs, as only this will demonstrate that it is actually a substance produced outside the organs—a substance which in no way can be produced by the organ itself. Such experiments have been started several times but could not be finished, as it is very difficult to obtain the proper material and to obtain it just at times when it is needed.

57 (1235)

The influence of diet on the heat production during mechanical work in the dog.

By GRAHAM LUSK.

[*From the Physiological Laboratory of the Cornell University Medical College in New York City.*]

The following table shows that when a dog runs at the rate of about 2½ miles an hour the heat production is almost exactly the same whether the dog has had no food or whether 70 grams of glucose have been administered. In the resting dog 70 grams of glucose would have increased the heat production six calories.

INFLUENCE OF FOOD AND MECHANICAL WORK ON THE METABOLISM OF DOG XV, WEIGHING 9.5 KG.

Results are given in hourly periods.

1917.	Calories.	R. Q.	Work in Meters Traveled.	Calories Above Basal per 1,000 M. Traveled.	Energy in Kgm. to Move 1 Kg. 1 M.
Jan. 5. Work, no food	61.3	0.80	4,140		
	61.0	0.77	4,080		
	61.1		4,110	10.5	0.470
Jan. 6. Work, glucose 70 g	62.8	0.96	4,080		
	62.1	0.97	4,100	10.8	0.485
	62.5				
Jan. 8. Basal	19.5	0.78			
	16.4	0.89			
Average	**18.0**	**0.84**			
Work, no food	60.0	0.94	4,410		
	67.1	0.83	4,190		
	63.5		4,300	10.6	0.475
Jan. 9. Work + glucose 70 g	64.8	1.04	4,350	10.8	0.485
Jan. 11. Work + meat 700 g	74.0	0.85	4,190	13.4	
	81.7	0.81	4,290	14.4	
	77.9		4,240		
Less results after "work, no food"	63.5				
Specific dynamic action of meat	14.4	which is 80 per cent. of the basal metabolism. The increase during the second hour is 100 per cent. of the basal metabolism.			

The experiment proves the economical use of carbohydrate during periods of work. On the contrary, when 700 grams of meat were given and the dog was compelled to run, the heat production was increased by that quota which would have been added from the specific dynamic action of the protein metabolized. The latter observation confirms Rubner; the first observation has never been reported. The principles are of importance in the proper arrangement of dietaries for those who execute mechanical labor.

58 (1236)

The cerebrosides of brain tissue.

By **P. A. LEVENE** and **C. J. WEST.**

[*From the Rockefeller Institute for Medical Research, N. Y.*]

Since the work of Thudichum, evidence has existed that there was more than one cerebroside. On the basis of physical properties and analyses, Thudichum distinguished two cerebrosides, phrenosin and kerasin. He had the correct intuition as to the points of difference of the two cerebrosides, and suggested that this difference was in the nature of one component, namely, the fatty acid. As far as he worked, the other components were identical in all cerebrosides. Unfortunately his method of separation was imperfect. Therefore, each fraction contained a large portion of the other. When the fatty acids were obtained, they analyzed approximately for a stearic acid, which Thudichum named *neuro stearic acid*. Subsequent workers improved the methods of purification particularly that of phrenosin; this permitted the isolation of the fatty acid, *cerebronic acid*. This acid was identified by Thierfelder as an hydroxy pentacosanic acid, $C_{25}H_{50}O_3$. The further work on cerebrosides brought nothing new as regards their structure. The attempt of Thierfelder to classify them on the basis of their sugar contents was futile. Finally, it was possible to isolate from a cerebroside corresponding to Thudichum's kerasin, a fatty acid of different structure from cerebronic acid, namely, lignoceric acid. This acid was isolated nearly simultaneously in three laboratories, but its relation to cerebronic and lignoceric acids was first established in our laboratory, and independently of us by Rosenheim. Since then, it has

been possible to judge of the purity of a cerebroside by the composition of the fatty acid fraction obtained on hydrolysis. This standard has been employed by us in testing the purity of the substance designated as phrenosin and kerasin.

By methods employed in different laboratories, it has been possible to obtain phrenosin in a fair degree of purity. Upon hydrolysis of phrenosin of the highest dextrorotation one obtains cerebronic acid. With the progress of purification the dextrorotation of phrenosin increases. On the other hand, the kerasin fraction acquires an increasing levorotation. Hence one can also be guided in purifying the kerasin by the magnitude of its levorotation. This method has been employed in our laboratory and also by Rosenheim; Rosenheim used in addition to this the selenite plate test. Guided by these tests, Rosenheim obtained substances which showed levorotation of -2 to $-3.5°$, and claimed them to be pure kerasin. On hydrolysis of these fractions he obtained only lignoceric acid.

Several years ago in our laboratory, hydrolyses were made on substances with the same and even higher rotatory power than those employed by Rosenheim. Using methods of hydrolyses different from the conventional method introduced by Thudichum, and used by all other workers, it was found that these fractions contained not only lignoceric acid but also cerebronic acid. From this it was concluded that kerasin had not, as yet, been prepared in a pure state. These results were not published because attempts to isolate kerasin in larger amounts by the methods then used, were not successful, and it was desired to obtain a more satisfactory method for preparation of the cerebroside.

It was thought that by the fractionation, not of the cerebroside mixture itself, but of an acyl derivative, one might obtain more satisfactory results. With this object in view the acyl derivatives of phrenosin and fractions corresponding to kerasin were prepared, as follows:

	M.	$[\alpha]_D^{20}$.	C.	H.
Hexa-acetyl phrenosin	41–43°	−11.08	65.31	9.27
Hexa-acetyl kerasin	54–56°	−16.46	67.00	9.95
Tri-benzoyl phrenosin	65–66°	+21.20	73.45	8.83
Tri-cinnamoyl phrenosin	69–70°	+21.72	73.22	9.45
Tri-*p*-nitrobenzoyl phrenosin	94–96°	+12.18	63.61	7.99

It was found that the benzoyl derivative of phrenosin was more insoluble in methyl alcohol, than that of kerasin. Indeed, by preparing the benzoyl derivative of a mixture with a rotation of ± 0.0 (in pyridine) one could separate this into two fractions, the more soluble of which had a rotation of − 2.25 to 3.2°, varying in different experiments. These rotations refer not to the benzoyl derivatives, but to the cerebrosides obtained after saponification of the acyl derivative. The substance with the highest levorotation (− 3.28°) obtained in this way corresponded to the one named by Rosenheim pure kerasin and the one we had in hand several years ago. Since that time the methods of hydrolysis have been improved; applying this for the analysis of new material, we again found that the fatty acid fraction contained both lignoceric and cerebronic acids. On the basis of this, we confirmed our original view that so-called kerasin has not yet been obtained in the pure state. On the other hand, the very gratifying results obtained by the benzoyl method carries confidence in the possibility of a successful separation of kerasin by repeated benzoylation of the levorotatory fraction of cerebrosides. The work in this direction will be undertaken as soon as a sufficient quantity of the cerebroside mixture will be prepared.

We wish to refer briefly at this place also to the problem of the sugar contents of cerebrosides. On the basis of the present assumption and their structure, the theory requires a value of 20 per cent. Employing Thudichum's method of hydrolysis Rosenheim reports a theoretical yield of sugar. This first seems mysterious to us. Control experiments on pure galactose carried out in our laboratory by G. M. Meyer showed that the sugar subjected to the treatment employed by Thudichum, Rosenheim and others suffered a very considerable destruction. Indeed, in the early part of the work, we could never obtain the required 20 per cent. For a time this seemed to be in the way of accepting the conventional theory of the structure of the cerebrosides. However, conditions of hydrolysis have been perfected in which the sugar undergoes little decomposition, and under such conditions the yield of sugar is as the theory requires, about 20 per cent.

59 (1237)

A consideration of the reduction of blood platelets in purpura.

By ALFRED F. HESS, M.D.

[*From the Department of Health, New York City.*]

As is well known, typical purpura is characterized by a marked diminution in the number of platelets; whereas the normal individual has about 300,000 platelets per cubic centimeter of blood, the purpuric individual has less than 100,000. Evidently this abnormal condition may be due either to a lack of formation of these cells, or to their increased destruction. A number of studies have been undertaken to decide this question, but no conclusive evidence has been brought forward.

The coagulation time of the plasma centrifugalized for fifteen minutes is normal in purpura or but slightly prolonged. If we centrifugalize plasma for two hours we find that, whereas the coagulation time in the normal case has been greatly prolonged—for example—from 8 to 18 minutes (Case 1, Table I), in the case of purpura, the centrifugation has frequently occasioned but little or no delay in coagulation (see Table I). This distinction may be interpreted as due to the fact that in the normal plasma we have removed all the platelet cells, whereas in the purpuric plasma, there is considerable platelet substance *in solution* which could not be removed by means of centrifugation.

This question can also be answered by means of employing a dilute solution of hirudin, an antithrombic substance, which combines with thromboplastic substances such as platelets. If we remove practically all the suspended platelet cells by means of prolonged centrifugation, we shall then be able to determine whether or not there is platelet substance in solution by attempting to neutralize it by the addition of hirudin. This test has been carried out in many normal as well as purpura cases. It has been found that whereas the addition of the hirudin solution to the centrifugalized normal plasma brings about considerable delay (in Case 3 of the table, from 8 to 30 minutes), in the purpuric plasma on the contrary, a considerably less degree of retardation is effected (for example, in Case 7—from 11 to 16 minutes). We

interpret such instances as indicating that the purpuric plasma contains dissolved platelet (thromboplastic) substance and that the deficiency of platelets in purpura is frequently due to a destruction of these cells. It becomes evident therefore that the explanation for the fact that we may have a normal coagulation time in purpura, in spite of greatly diminished platelet count, can be accounted for on the ground that there is no true lack of platelet substance; the deficiency is merely apparent, the total amount of platelets in solution and in suspension equalling the normal. In some cases inhibiting coagulative factors also play a rôle.

In cases of purpura there is frequently a hemolytic substance in the plasma which renders it exceedingly difficult to find compatible donors for transfusion. It is possible that this lytic substance is associated with the function of the spleen. We know such to be the case in pernicious anemia and hemolytic icterus in regard to the destruction of the red cells. It has furthermore been established that the removal of the spleen, both in man and in animals, brings about a definite increase in the number of blood platelets. It would therefore seem worthy of trial to perform a splenectomy, immediately preceded by a blood transfusion, in severe cases of purpura where extreme therapeutic measures—repeated transfusions—have been resorted to in vain.

TABLE 1.

SHOWING DIFFERENCE OF EFFECT OF PROLONGED CENTRIFUGATION AND OF THE ADDITION OF HIRUDIN SOLUTION UPON NORMAL AND PURPURIC PLASMA.

Type of Case.		Coagulation Time.		No. of Hours Centrifugalized.	Coag. Time after Adding Hirudin Solution.
		After 15 Min. Centrifugation.	After Prolonged Centrifugation.		
Normal	(1)	8	18	2	
	(2)	9	18	2	
	(3)	4	8	1	30
	(4)	1			28
Purpura	(5)	14	14	2	
	(6)	26	26	2	
	(7)	10	11	1	16
	(8)	18			21
Hemophilia (9)		30	94	1	

60 (1238)

The effects of ageing upon germ cells and their development.

By A. J. GOLDFARB.

[*From the College of the City of New York, N. Y.*]

For several years experiments have been made with the germ cells of the tropical sea urchins *Toxopneustes* and *Hippanoe*, and the northern urchin *Arbacia*. The preliminary experiments were made with a view towards obtaining experimental conditions that were optimum and that gave the least variability for freshly removed eggs and sperm from freshly collected sea urchins. Surprisingly large differences were observed among the eggs from different females. These differences involved (1) the size of the eggs, (2) the presence of the jelly layer, (3) rate of membrane formation, and (4) cleavage.

By means of one or more of these criteria it was possible to grade the different freshly collected females according to the physiological condition of their eggs. Eggs of similar physiologic condition showed a minimum variability and the highest correlation with respect to these variants.

When eggs and sperm were removed from their respective bodies and kept under optimum laboratory conditions, the same changes that had begun within the bodies of the sea urchins continued outside of the body.

With increasing age outside of the body the eggs showed progressive changes in size, in loss of jelly, in retarded membrane formation, in decreased total cleavage and decreased rate of cleavage, etc. And the exact degree of change in these regards was ascertained for different intervals up to the death of the eggs.

Still other changes were consequent upon ageing of the eggs, which suggested the nature of the chemico-physical agencies involved in the ageing process: namely, agglutination, fusion of two or more eggs, separation of the blastomeres, and irregular cleavage.

These changes suggested that the excess free HO ions in the sea water was one agency and probably a very important one in causing the dissolution of the jelly, the changes in permeability of the cortical layer of the eggs, the changes in size, and all the

other changes mentioned above, that follow upon long exposure to the free HO ions.

If these ions are responsible for the ageing one should be able to age eggs precociously with hyperalkaline sea water and on the contrary one should be able to retard ageing by rendering sea water neutral. Such experiments were made and made repeatedly. Eggs were aged precociously and showed all the physiologic and developmental changes which I have shown were characteristic of overripe eggs, and on the contrary ageing was retarded by removing the excess HO ions.

The longevity of the eggs was increased either by a reduction of respiration by KCN as proposed by Loeb, Lyon and others, or by the elimination of the excess free HO ions of sea water.

Other changes in ageing eggs were studied, such as the metabolism of the eggs, and the later developmental changes, but these studies will be reported later.

61 (1239)

The influence of thyroidectomy on the blood sugar.

By N. W. JANNEY and V. I. ISAACSON.

[*From the Chemical Laboratory of the Montefiore Home and Hospital for Chronic Diseases, New York City.*]

Considerable uncertainty exists with regard to the relation of thyroid function to carbohydrate metabolism. Some writers report a decrease, others, an increase of alimentary sugar tolerance after thyroidectomy in the dog. This confusion is due to several causes. Injury to the parathyroid bodies, which probably exert a different effect on metabolism from that of the thyroid, has frequently not been excluded. Previous experiments are open to other technical criticisms. The observations have been chiefly confined to urinary examinations.

Accordingly, a study of the blood sugar was made before and after thyroidectomy in a series of dogs. The operations were performed by Dr. J. E. Sweet. At least two parathyroids were isolated in each case. The wounds healed aseptically, and the dogs remained free from tetany. The blood sugar after thyroidec-

tomy showed a marked decrease, averaging about 25 per cent. less than the normal value. The feeding of 6½ grams of glucose per kilo in 40 per cent. solution before the operation to fasting animals showed the normal hyperglycemia to last three hours. After thyroidectomy, glucose fed under the identical experimental conditions, failed to raise the blood sugar to the level attained before the operation. The increase persisted however for an average of five hours. The urine remained free from glucose both before and after the operation with a single exception.

We have observed hypoglycemia in cretinism. It is present in myxedema, in Addison's disease, and after removal of the suprarenal bodies. Evidence is accumulating showing that a persistent low sugar content is a sign of insufficient internal secretion.

Quite aside from the doubtful question of whether increased alimentary tolerance to glucose can be demonstrated by an examination of the urine, these experiments show that there is less tendency to hyperglycemia on carbohydrate ingestion after thyroidectomy. It seems therefore, that the removal of thyroid tissue might lower the blood sugar also in diabetes mellitus, possibly with beneficial results.

62 (1240)

Further observations on the influence of diet on the toxicity of sodium tartrate.

By WILLIAM SALANT and A. M. SWANSON.

[*From the Pharmacological Laboratory, Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.*]

In a previous publication from this laboratory[1] it was stated that the toxicity of sodium tartrate might be modified by diet. Rabbits that were fed carrots resisted larger doses of the tartrate than those which received oats and cabbage. The investigation of the effect of diet on the toxicity of this substance was resumed recently. The observations were made on rabbits and on cats with a large number of diets which were given some time previous to the subcutaneous injection of tartrate. Striking differences in toxicity were observed. When young carrots were fed four days

[1] Salant and Smith, *Am. J. Physiol.*, 1914, *35*, 239.

before the tartrate was given, the dose survived was 3.0 grams per kilo. One rabbit only developed albuminuria when this dose was given. In one series of four rabbits three survived such a dose. In another series with 3.5 grams per kilo, one survived and three died, two in 12 and 36 hours and one in 6 days. The resistance was likewise very marked when carrot leaves were fed 4 to 11 days before injecting tartrate, but was less than in the case of young carrots, the minimum fatal dose being about 2.5 grams per kilo. The duration of life in this case was 2 to 5 days, the rabbit dying, however, without developing albuminuria. The toxicity when mature or winter carrots were fed was on the contrary considerably greater, the fatal dose being 1.25 to 1.5 grams per kilo.

Exactly the same results were obtained with sweet potatoes as with carrot leaves, as 2.5 grams per kilo likewise proved to be fatal, while a dose of 2.0 per kilo failed to produce any nervous symptoms or renal irritation. Sodium tartrate proved to be most toxic when the diet consisted of oats and cane sugar, glucose, or levulose. When 0.5 gram per kilo was injected subcutaneously on this diet, seven out of 8 rabbits died after 2 to 13 days. Symptoms of renal irritation and nervous disturbances were noticed in these experiments. The toxicity on hay, cabbage or oats was about the same in each case and was approximately twice that on oats and sugar, or about one fourth that on young carrots. The resistance to sodium tartrate on a diet of sugar beets was about half that on young carrots. Experiments with tartrate on cats that received different diets failed to show any marked difference, but when starved for eight days the toxicity was increased about 40 per cent.

63 (1241)

A note on the parenteral administration of starch.

By C. E. KING.

[*From the Laboratory of Physiology, University of North Dakota.*]

Very little work has been done on the question of the production of a protective amylase after the parenteral administration of starch. Most observers agree that the blood normally contains

a starch-splitting enzyme. There is also abundant evidence that the term amylase is applied to a variety of starch-splitting enzymes characterized chiefly by the extent of the hydrolysis, such as the production of soluble starch, dextrins, and reducing sugars. We have found that the blood of a given animal may vary considerably from day to day, sometimes producing dextrins slowly and reducing sugars rapidly, or dextrins rapidly and reducing sugars slowly. It is evident that the study of the formation of a protective amylase after parenteral introduction of starch calls for a quantitative study of at least three phases of starch hydrolysis.

We have at hand data on seven dogs. These animals have received both single and repeated intravenous injections of soluble starch. No constant or significant increase in the amylolytic activity of the blood has been found. There is, however, a great increase in the amylolytic power of the urine on the day following the injection of the starch, the increase being shown by a more rapid production of both dextrins and of reducing sugars.

In the interpretation of these results it appears possible that there is an increased production of amylase after the injection of starch, but the kidneys eliminate it so rapidly that there never is present in the blood enough of the excess over the normal to be detected by any of our present methods. On the other hand the amylase may be present in the blood in an inactive state, perhaps in combination with some colloid, and on the introduction of a suitable substrate this combination is broken up, and at least a part of the enzyme eliminated by the kidneys before it can recombine.

Experiments are now in progress for the purpose of determining whether there is an actual increased production, and if so, where it takes place, and also to throw more light on the second hypothesis mentioned above.

ABSTRACTS OF THE COMMUNICATIONS, PACIFIC COAST BRANCH.

Sixteenth meeting.

San Francisco, California, February 10, 1917.

64 (1242)

The development of pieces of diemyctylus embryos.

By S. J. HOLMES.

[*From the Department of Zoology, University of California.*]

In a series of experiments performed on pieces of embryos of the newt *Diemyctylus torosus* care was taken to eliminate all sources of infection. The jelly surrounding the embryos was washed in a solution of mercuric chloride and then in sterile distilled water and transferred to sterile Ringer's solution in which the embryos were liberated. The embryos were then cut by means of a sharp razor into pieces of various sizes which were kept in sterile Ringer's solution. Even relatively small pieces showed developmental changes, and pieces from the neck region sometimes developed outgrowths which very closely resembled the gill filaments of the normal larvæ. In embryos cut in two just behind the gill region the posterior part which was kept alive for some months underwent a course of development strikingly like that of the normal embryo. The outgrowth of the tail, the development of the transparent median tail fin or keel, the formation of pigment cells, and the differentiation of tissue cells in general occurred in a perfectly normal manner so far as could be observed. These pieces increased in size, doubtless through the absorption of water, and became relatively transparent as the yolk granules in the cells were assimilated. In nearly all of these pieces the heart was entirely absent. Correlated with this was a complete absence of peripheral blood vessels. In the controls these were well developed and blood could be seen rapidly streaming through them, but, although the pieces developed far beyond the stage at which these blood vessels normally appear, they showed at no time any trace of these peripheral vessels. Sections, however, showed that the aorta and some of the larger veins were more or less imperfectly developed.

In several cases the anterior limbs budded out and differentiated in a normal manner, showing all the digits completely formed; such larvæ would swim about in a lively manner when they were stimulated. In fact the posterior pieces of these embryos developed much beyond the stage at which normal lavræ begin to take food, but whether lack of food precluded them from developing farther cannot as yet be stated.

A definite circulatory system is not necessary for the development of legs, gill filaments, the growth of the tail or the differentiation of internal organs up to quite a late period of larval life. The absence of the thyroid, thymus and pituitary body seems to produce no marked changes up to the period in which the legs and gills are well developed.

65 (1243)

On "racemized" casein.[1]

By CARL L. A. SCHMIDT.

[*From the Rudolph Spreckels Physiological Laboratory and the Hearst Laboratory of Pathology and Bacteriology of the University of California.*]

The remarkable observation made by Dakin and Dudley[2] that "racemized" casein is not attacked by pepsin, trypsin, or erepsin, is apparently excreted unchanged when injected subcutaneously or given by mouth to a dog, and is unaffected by bacteria, would indicate that the chemical structure of casein had been decidedly altered during the process of "racemization." Dakin[3] suggests that the process is a tautomeric change of the keto-enol type in the manner $> CH—CO—NH— \rightleftarrows > C = C—OH—NH—$ and that in the case of "racemized" casein this change must be complete for all the groups in the protein molecule, otherwise a point of attack for enzymes would be afforded and a partial splitting might occur. If the ketol-enol conversion be complete, the apparent discrepancy pointed out by Kober that all racemic peptides are attacked by erepsin would not necessarily be a real one, since

[1] Aided by a grant from the George Williams Hooper Foundation for Medical Research.

[2] Dakin, H. D. and Dudley, H. W., *J. Biol. Chem.*, 1913, 15, 271.

[3] Dakin, H. D., *J. Biol. Chem.*, 1912–1913, 13, 357.

the tautomeric change suggested by Dakin is not racemization in the ordinary meaning of the term. The discrepancy as to the number of end amino groups in casein, as indicated by the optically active amino acids found by Dakin, is as Kober[1] shows, fatal to Dakin's theory.

Anticipating the probability that a substance which is so changed as to be unaffected by enzymes and bacteria might also be changed in its antigenic properties, Ten Broeck,[2] at Dakin's suggestion, carried out experiments with "racemized" egg albumin and found that the substance was non-antigenic as shown by the anaphylaxis, precipitin and fixation tests. Underhill and Hendrix,[3] on the basis of fall of blood pressure and retardation of blood clotting, find that "racemized" casein, egg-albumin and zein are non-toxic, but yield toxic products on partial hydrolysis with acid. The "racemic" proteoses, with the curious exception of zeose, are likewise toxic. It appears, then, that the "racemic" substances are not entirely without action on the body when introduced parenterally.

The "racemization" of proteins appears to throw some light on the relationship of structure to antigenic property. Based on "racemization" experiments, the work of Dudley and Woodman[4] would indicate that casein from the sheep differs from casein obtained from cow's milk.

In connection with other work being done on the relationship of protein structure and antigenic property, it appeared to the writer of importance to fill in the gap which in the particular case of casein is still missing, and to determine whether racemized casein is likewise non-antigenic. "Racemic" casein was prepared essentially according to Dakin's method, except that for the purpose of drying the product readily, it was washed with alcohol and ether which removed the larger portion of the caseose with which it is usually contaminated. In preparing solutions of both casein and the racemized product, sufficient alkali was added to make the solution neutral to litmus. The rabbits received injec-

[1] Kober, P. A., *J. Biol. Chem.*, 1915, 22, 433.
[2] Ten Broeck, C., *J. Biol. Chem.*, 1914, 17, 369.
[3] Underhill, F. P. and Hendrix, B. M., *J. Biol. Chem.*, 1915, 22, 453.
[4] Dudley, H. W. and Woodman, H. E., *Biochem. J.*, 1915, 9, 97.

tions over a period of a month with the substances and in the manner and dosage as follows:

"Racemized" casein, No. 869—One 100 mg. dose intraperitoneally and seven 100 mg. doses intravenously.

No. 670—Five 100 mg. doses intraperitoneally and three 100 mg. doses intravenously.

Nos. 849 and 850—Five 100 mg. doses intraperitoneally and four 100 mg. doses intravenously.

No. 871—Previously immunized with casein and high titer serum obtained.

Eight days after the last injection the animals were bled and fixation experiments carried out in the usual manner, using one fourth of the minimum inhibiting dose of antigen and 1.5 units of alexin. The serum of animals immunized with casein gave positive tests with quantities of serum less than 0.02 cc. (titration not carried to limit); the sera of animals injected with racemized casein were negative in doses which themselves were not inhibitive on omission of the antigen. Similar tests using the heterologous antigen showed no fixation, showing that "racemized" casein is not identical with casein.

The observation made by Robertson[1] that when a current is passed through a solution of casein a precipitation of casein occurs on the anode, was found to be also true for the racemized product. It must therefore be dissociated. But, as pointed out to me by Dr. Robertson, if Dakin's theory of the ketol-enol conversion be true, we would be compelled to assume that the "racemic" protein dissociates in a different manner than the now generally accepted theory proposed some years ago by Robertson.[2] According to his theory the dissociation of the protein salts with inorganic bases and acids is accomplished in the manner

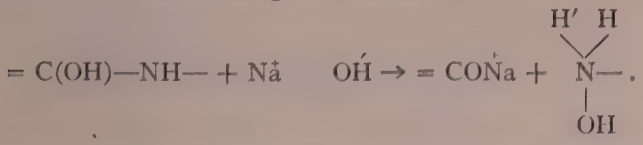
$$-COH = N- + \overset{+}{N}a \qquad \overset{-}{O}H \rightarrow -CO\overset{+}{N}a + \begin{matrix} H'' \\ | \\ N- \\ | \\ OH \end{matrix}.$$

If the "racemization" of casein is due to the oscillation of a labile hydrogen atom attached to the carbon atom, we must either assume, if the substance dissociates in the usual manner, that hydro-

[1] Robertson, T. B., *J. Phys. Chem.*, 1911, 15, 179.

[2] Robertson, T. B., *J. Phys. Chem.*, 1911, 15, 521.

gen is split off, or if hydrogen is retained, we must have two protein ions each having only a single latent valency capable of neutralizing a unit charge as the following will indicate:



Such a case has not as yet been shown, though the possibility may exist. It is also possible that a change other than the ketol-enol tautomerism takes place.

SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

Eighty-second meeting.

Presbyterian Hospital, March 21, 1917.

President Gies in the chair.

66 (1244)

Some observations concerning chicken bone marrow in living cultures.

By RHODA ERDMANN.

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Tower and Herm[1] presented recently before the Society for Experimental Biology and Medicine some ideas concerning the origin of the mammalian (cat) and avian (chicken) blood cells. These authors were led by their observations on bone marrow in living cultures to the following conclusions:

1. The mammalian red blood corpuscle is a nuclear bud which escapes into the circulation as the true red cell.

2. The mammalian normoblast and the red corpuscle of the bird are the product of intranuclear activity and are phylogenetically identical.

3. Phagocytosis of red cells by the giant cells (megakaryocytes) in normal blood-forming tissues is by no means common. The true process is undoubtedly the manufacture of red cells and not the destruction of them.

My own observation of *chicken* bone marrow in living cultures led to some conclusions which are not in harmony with the quoted statement. I studied the bone marrow of *chicken*; therefore, my remarks are based only on observations on the bone marrow cells of this animal.

[1] Tower and Herm, 1916, PROCEEDINGS OF THE SOCIETY OF EXPERIMENTAL BIOLOGY AND MEDICINE, 1917, Vol. XIV, pp. 61–52.

I could observe budding of red blood corpuscles after the first day of cultivation. Buds with and without nuclei appeared, also the rapid division of erythroblasts could be noted. The budding off of either small nucleated or non-nucleated cells cannot be a progressive process in the chicken because the avian red blood corpuscle is nucleated. Therefore the analogous observation of Tower and Herm with its conclusion that the mammalian red blood corpuscle is a nuclear bud which escapes into the circulation as the true red cell, loses its convincing power; probably the budding is a reaction of the normoblast to the change of its media, as it is also observed in amebae as soon as they are under unfavorable conditions and can be produced experimentally. The mono- and polynuclear eosinophil leucocytes show in living cultures the same tendency to divide rapidly into nucleated *small* cells or non-nucleated components in living cultures. The bud-forming capacity and the tendency to divide rapidly seems to be a general behavior of blood cells in living cultures.

Another phenomenon to produce non-nucleated cells observed in living cultures is the following: the nucleated blood corpuscle loses its nucleus by *ejection* of chromatin, this process resembles the formation of Cabot's bodies in experimental anemia or the anemia of man (Juspa[2]).

These two processes seem to be *degenerative in the chicken.* If these two phenomena are observable in the chicken the question arises, can the mammalian normoblast be capable of losing either its nucleus by the budding off process or by ejection of the condensed original nucleus. The authors identify strongly in their second thesis the mammalian normoblast and the red corpuscle of the bird, it may be therefore possible that they have not observed the ejection of the mammalian normoblast nucleus.

I can agree with the authors that phagocytosis of true *megacaryocytes* (giant cells) is by no means common in normal bone marrow tissues. But still the most striking feature in *my cultures* was the phagocytosis of a kind of "Riesenzellen." But these "Riesenzellen" are not the usual multinucleated cells of the bone marrow; (megacaryocytes) this name includes cells which have

[2] Juspa, 1913–14, *Folia Haemat.*, Bd. XVII, II Teil, pp. 429–441.

first been observed in the bone marrow by Foot[3, 4] and thought by him in his first publication to be of mesenchymal origin and in the second publication of lymphocytic origin. These "giant cells" can be either changed fat cells or elongated vacuolized connective tissue cells, or even enlarged myelocytes. They are able to phagotize, to store fat, to divide into smaller forms—the so-called cell culture type, small forms with nuclei, the chromatin of which is arranged on a fine network. The formation of "giant cells" characterizes the first period in the history of bone-marrow in living cultures. After five or eight days, cultivation, the "giant cells" have cleaned up the debris of the dying cells (blood corpuscles, fat cells, and large mononuclear lymphocytes). In the second period the remaining cells adjust themselves to the continued life in tissue cultures. Cell types of the small lymphocyte type with vesicular nuclei appear, which later are transformed into different types of connective tissue cells—not exactly resembling the connective tissue cells in the outgrown animal—but closely resembling the mesenchymal cells of the embryo.

The production of the cells of the second period can be accelerated by washing the original bone marrow particle in plasma. After the plasma has been renewed three or four times, blood corpuscles and the lymphocytes which had been from the beginning in the meshes of the bone marrow, have been left in the media and only those cells close to the bone marrow network are transferred to the new culture medium. In cultures prepared in this way, we can observe small cells of lymphocytic character which can store fat, phagotize and adopt all shapes of connective tissue cells—but no formation of *blood* corpuscles or large mononuclear lymphocytes can be observed. This proves clearly that after the already preformed "ripe cells" are disposed of, no new formation of blood corpuscles takes place in the living cultures. It may be that the lack of oxygen prevents the appearance of red blood corpuscles. The conditions in tissue cultures do not seem to allow the stem cell to show its dualistic character. It does not form blood corpuscles, but forms only the different elements of *connective* tissue.

[3] Foot, *Beitr. z. path. Anat. u. z. allg. Path.*, 1912, Bd. 53, pp. 446–447.
[4] Foot, *Jour. of Exp. Med.*, 1913, Vol. XVII, pp. 44–60.

The above mentioned authors do not state in their preliminary paper how old their cultures were when they made their observations and if they have distinguished between cultures of fat containing bone marrow, nearly fatless bone marrow and bone marrow with large amounts of red blood corpuscles. All these facts may alter the conclusions because if the bone marrow contains a large amount of fat then many "Riesenzellen" are present and phagotization can be observed in a very considerable degree. If fatless bone marrow is used, phagotization appears to go on in a remarkable degree only in the second outlined period of culture life, because only then the cells near the bone marrow network begin to migrate in the plasma clot, to phagotize and to assume different types of connective tissue cells.

67 (1245)

On the isolation of streptococci from rabbits.

EDGAR T. H. TSEN (*by invitation*).

[*From the Department of Bacteriology of the College of Physicians and Surgeons, New York.*]

In our work for the purpose of investigating the relation of streptococci to poliomyelitis as claimed by Dr. E. C. Rosenow, we have made cultures of the brains of 6 monkeys and 20 rabbits. Our technique was as follows:

The animals were etherized just before death and, when anesthetized, were fastened to an autopsy-board—abdomen downward. With animals that died during the night this part of the procedure could not, of course, be carried out. Our purpose was to make sure, whenever possible, that any organisms cultivated from the brains were not post-mortem invaders, but were present during the life of the animal. A median incision was made through the skin over the skull running from the tip of the nose to the back of the neck, and the skin dissected back on both sides of the head. The skull was disinfected with tincture of iodine, and the head and body covered with 3 layers of gauze soaked in lysol. A small hole was made in the gauze so as to expose the upper

part of the skull. The skull was next opened, and, to further insure sterility, the surface of the brain was seared with a scalpel. A big piece, and sometimes one-half of the brain was removed with a pair of long forceps and then immediately put into a Rosenow sterile air chamber, where it was emulsified. (The chamber was sterilized in the hot-air sterilizer for one hour at 160° C.) In some cases it was possible to remove considerable parts of the brain while the animal was still alive. The emulsion was poured into a large tube from which different media were inoculated with sterile pipettes. The tubes were examined after two days and, if they were found to be sterile, were again examined two or three days later. The cultures were all kept at 37.5° C.

The media used were plain broth, ascites broth, glucose broth, glucose ascites broth, plain broth with tissue, ascites broth with tissue, glucose broth with tissue, glucose ascites broth with tissue, glucose agar, and glucose ascites agar. The amount of glucose in the broth was 0.2 per cent. and that in the agar 1 per cent. The reaction of all the media was 0.6 + to 0.8 +.

A part of the tubes inoculated in each case was prepared by methods simulating as exactly as possible those employed by Dr. E. C. Rosenow.

All the six monkeys were inoculated with glycerinated poliomyelitis virus—4 intracerebrally, 1 subcutaneously, and 1 both intracerebrally and intraperitoneally. Both anærobic and ærobic cultures of the brain and cord emulsions were made on ascites glucose agar slants at the time of inoculation. We found no growth in any of the tubes. These monkeys were found dead or were etherized from 4 to 16 days after inoculation. Those 4 which were inoculated intracerebrally showed typical changes of poliomyelitis and the other two did not.

From all these 6 monkeys we have isolated streptococci. On November 12, 1916, two rabbits were inoculated intravenously with a 20-hour culture of streptococci isolated from the brain of a monkey which showed typical changes of poliomyelitis. Before inoculation this culture was stated by Dr. Rosenow to be similar to his. Rabbit 1151 was inoculated with the growth from 22.5 c.c. of ascites glucose broth suspended in 1.5 c.c. of sterile salt

solution, and rabbit 1152 with that from 30 c.c. of the same medium suspended in 2 c.c. of sterile salt solution. Cultures of the bacterial suspension were made at the time of inoculation, and the streptococci were found to be alive and free from contamination. On December 2, 1916, rabbit 1151 was again inoculated with a bigger dose. These two rabbits are still in normal condition today—more than 4 months after inoculation.

Of the 51 rabbits with which we have worked 11 were inoculated with glycerinated poliomyelitis virus—7 died and 4 are living; 9 were inoculated with the brain emulsions of rabbits that died after inoculations with poliomyelitis virus—2 died and 7 are living; 6 were inoculated with streptococci from monkeys—3 died and 3 are living; 13 were inoculated with streptococci from rabbits that died after inoculations with poliomyelitis virus—5 died and 8 are living; 3 were inoculated with streptococci from rabbits that died after inoculations with streptococci—1 died and 2 are living; 2 were syphilitic, and 7 were normal.

Of the 18 rabbits that died after inoculations with poliomyelitis virus or streptococci, none showed any of the changes usually thought characteristic of poliomyelitis of man and monkey. Nevertheless, we have isolated streptococci from the brains of all the 11 on which we have made autopsies. Seven were inoculated with poliomyelitis virus.

We have also isolated streptococci from the brains of the two syphilitic rabbits and 5 out of the 7 normal rabbits. Or in other words, only the brains of two normal rabbits remained sterile.

It therefore appears from our experiments that streptococci are probably saprophytic microörganisms in the normal body which, under conditions of lowered resistance in the course of disease from other cause, acquire the power of more extensively invading the tissue. They do not seem to have any etiological relationship to poliomyelitis.

We are continuing this work with more special attention to a study of the tissue sections and of the cultivation of the globoid bodies.

68 (1246)

The non-protein nitrogen of blood: 1. The removal of the protein. 2. The estimation of creatine.

By ISIDOR GREENWALD.

[*From the Harriman Research Laboratory, Roosevelt Hospital, New York.*]

In a previous publication,[1] the author described a modification of the method of Folin and Denis[2] for the determination of non-protein nitrogen in blood. Trichloracetic acid was used to precipitate the protein and the trace that remained in the filtrate was removed by shaking with kaolin. It was shown that the nitrogen of an amino-acid mixture added to blood could be completely recovered by this method and that no nitrogen was split off from proteins by this treatment. Bock[3] recently described another method for obtaining protein-free filtrates from blood. He coagulated the protein by running the blood into boiling 0.01 *N* acetic acid, evaporated the filtrate to a small volume, precipitated most of the remaining protein with trichloracetic acid and removed the last traces with kaolin. It seemed that it should be possible to remove the protein from the filtrate from the coagulum by means of kaolin, directly, without the use of trichloracetic acid. This was found to be the case. Kaolin is added to the filtrate from the coagulum, shaking the mixture thoroughly, until the foam, which is at first voluminous and persistent, becomes scanty and comparatively evanescent. One drop of glacial acetic acid for each 100 c.c. of fluid is then added in order to agglutinate the kaolin and the mixture is then filtered. The filtrate is protein-free, so nearly as may be determined by the usual tests. It may be evaporated to small volume (less than one tenth that of the original blood) either at atmospheric or reduced pressure without foaming. Determinations of the nitrogen in such filtrates agree with those obtained by the trichloracetic acid-kaolin method. However, both methods are made inaccurate through the removal

[1] Greenwald, *Journal of Biological Chemistry*, 1915, 21, 61.

[2] O. Folin and W. Denis, Ibid., 1913, 11, 527.

[3] Bock, Ibid., 1917, 28, 357.

by the kaolin of nitrogenous substances other than protein. This is most marked with substances of a basic nature, including diamino-acids and ammonia (present as ammonium chloride), and is absolutely quantitative with creatinine, even with amounts several times as great as those in the filtrates from the blood coagula. In the presence of trichloracetic acid, absorption appears to be less marked. The omission of kaolin after the precipitation of the protein by means of trichloracetic acid apparently leads to more accurate figures for the total non-protein nitrogen of the blood. These are about 3 mg. per 100 c.c. of blood higher than those obtained with the use of kaolin. According to Folin and Denis,[1] the results obtained by their meta-phosphoric acid method agree very closely with those obtained by their methyl alcohol method. Since the latter has been shown to give too low results,[2] it would seem that meta-phosphoric acid, also, does not yield all the non-protein nitrogen to the filtrate. Gettler and Baker[3] used hydrochloric acid and mercuric chloride for the precipitation of protein and obtained higher values for the non-protein nitrogen of the blood than have other observers. Comparison of the two methods in this laboratory[4] failed, in the few experiments made, to reveal any marked difference in the results obtained by the use of hydrochloric acid and mercuric chloride and by the use of trichloracetic acid and kaolin. Gettler and Baker do not direct the use of sodium sulfide or other precipitants for the mercury before the distillation. Unless the mercury is removed or precipitated as sulfide, the results be as much as 50 per cent. too low.

Although creatinine, under the same conditions, is quantitatively removed by kaolin, *creatine is not absorbed at all*, either from dilute aqueous solution or when added to blood. This offers a method for the estimation of creatine in the blood. The filtrate from the kaolin is treated with a small volume of *N* hydrochloric acid and evaporated to approximately that volume. (In most of the experiments, a volume of filtrate equivalent to 50 c.c. of blood was evaporated to 5 or 10 c.c. after the addition

[1] Folin and W. Denis, Ibid., 1916, 26, 491.

[2] I. Greenwald, loc. cit.; Bock, loc. cit.

[3] A. O. Gettler and W. Baker, Ibid., 1916, 25, 210.

[4] I. Greenwald, loc. cit. (page 64) and unpublished expts.

of 10 c.c. of *N* hydrochloric acid.) This is then neutralized with sodium hydroxide and creatinine is determined in the usual manner. When only small amounts of blood are available, 1 c.c. of acid is used and, after evaporation and neutralization, the solution is diluted to 25 c.c. with saturated picric acid and the determination is made by Folin's micro-method. The amount of creatine thus found is about 4 mg. per 100 c.c. of blood. Added creatine is recovered quantitatively, showing that there is no conversion to creatinine in the process of coagulating the protein. No claim is made that the substance reacting with picric acid and sodium hydroxide under these conditions is creatinine derived from the creatine of the blood. However, it is apparently not formed by the condensation of glucose, urea and uric acid. A solution containing 350 mg. glucose, 125 mg. urea and 5 mg. uric acid was evaporated with 10 c.c. of *N* hydrochloric acid. There was an apparent creatine content of 0.1 mg. Smaller amounts of urea and glucose gave no perceptible amounts of chromogenic substance.

69 (1247)

Anaphylaxis in the dog.

By RICHARD WEIL.

[*From the Department of Experimental Medicine in the Medical College of Cornell University, New York.*]

1. Dogs in severe anaphylactic shock have been bled to death, and the blood has been used to transfuse normal dogs. No symptoms of any kind have been produced. Hence the conclusion is drawn that the symptoms of anaphylaxis are not due to the presence of toxic substances in the blood.

2. The liver of sensitized dogs has been perfused with normal blood by means of anastomosis of the portal vein with the carotid of another dog. The blood, as it flows from the inferior cava, clots within a few minutes. If the antigen (horse serum) is injected into the connecting tubing, the blood in the cava soon becomes less coagulable, or quite incoagulable. The conclusion is drawn that the incoagulability of the blood is due, at least in part, to the reaction of the sensitized liver cells to the antigen.

3. Peptone shock has long been known to resemble anaphylactic shock closely, and the inference has generally been drawn that the latter is due to the production and circulation of peptone-like bodies. But the transfusion experiments above described do not bear out this theory. The suggestion is made that these two syndromes coincide for the reason that both alike result from a reaction of the liver. Phosphorus and chloroform, both hepatic poisons, also produce entirely similar changes in the chemistry and coagulability of the blood. Peptone is known to protect sensitized animals against anaphylactic shock. I have found that phosphorus or chloroform poisoning exerts a similar effect. The conclusion is drawn that the liver may be partially exhausted by any of these methods, and will not then react as acutely in anaphylactic shock.

4. Anaphylaxis in dogs is a cellular phenomenon, due chiefly, if not wholly, to the reaction of the sensitized liver cells.

5. It is suggested that the fall of blood pressure in anaphylactic shock may indirectly be due to the liver. That organ is found to be enormously congested, and it is conceivable that the animal is "bled to death" into its own liver. Or, the drop in pressure may be a vaso-motor reflex, comparable to the Goltz phenomenon, initiated by the acute hepatic shock of the reaction.

70 (1248)

The response of the respiratory mechanism to rapid changes in the reaction of the blood.

By JOHN P. PETERS, JR., M.D. (*by invitation*).

[*From the Medical Clinic, Presbyterian Hospital, and the Coolidge Fellowship for Medical Research, Columbia University, New York.*]

For the past year we have been studying the reaction of the blood by two parallel methods: the Fridericia method for alveolar CO_2 and the Van Slyke determination of the carbon dioxide combining power of the blood. We believe that this combination offers a simple method for the study of the state and the reaction of the respiratory mechanism.

In no normal person have we found a disagreement of over 10 per cent. in the ratio of alveolar/plasma CO_2. Discrepancies observed seem to fall into two large classes: (1) Those due to a mechanical interference with the gaseous exchange, and (2) those due to changes in the control of the respiratory mechanism.

The first class is best illustrated by patients with cardiac dyspnea or very great dimunition of the pulmonary capacity. In these the alveolar is invariably lower than the plasma reading. If compensation is established the two again come into agreement. This, we believe, is due to an impairment of the lungs that renders the excretion of CO_2 more difficult. The body overcomes this, in response to stimulation of the respiratory center by the retained CO_2, with a greater pulmonary ventilation. In this way a pressure difference is established between the carbon dioxide tension in the blood and in the alveoli, sufficient to compensate for the impairment in the lungs. The increased minute volume, the intolerance to carbon dioxide in the inspired air, the dyspnea and the disproportionate response to exercise are all expressions of the same thing.

The second class is best illustrated by the discrepancies observed after rapid changes in the reaction of the blood. The alveolar air tends to lag behind the plasma in its response. This is susceptible of an easy explanation during a sudden increase of the H-ion concentration of the blood, when the demand on the respiratory mechanism is overwhelming and the lungs are flooded with CO_2. We have been unable to study any patients who have developed spontaneously a persistent acidosis, to determine the time relations of the change. We have found that adrenaline given intramuscularly to normal and diabetic persons produces an acidosis, the curve of which is somewhat more rapid than that of the hyperglycemia. After adrenaline and after exercise the return to normal is very rapid. In none of our cases did the alveolar CO_2 fall as far as the plasma CO_2.

A similar retardation of the alveolar response is found after rapid alkalinization of the blood. This was first noted after the administration of carbonates for therapeutic purposes, but has since been determined in several cases of diabetic acidosis of various degrees that have recovered without bicarbonate. In no

such case have we failed to find a discrepancy. The abnormal alveolar/plasma ratio persists for some days. This would make it seem unlikely that the disturbance was due to the fixation of CO_2 by the accession of alkali to the blood. It persists after all obvious hyperpnea is gone, so that an increase of the sensibility of the respiratory center can not be offered as a definite explanation as yet. The fault is not in the choice of the Fridericia method as it has also been observed with the Plesch-Higgins method. It seems unlikely that it is dependent on personal factors because of the absolute constancy of its relation to rapid changes in blood reaction and its absence at all other times. We are continuing studies to determine the cause of the phenomenon.

We believe that alveolar methods do not indicate accurately the reaction of the blood after recovery from acidosis. We also suggest that the value of respiratory quotients at such times may be questionable.

71 (1249)

Active immunization with sensitized and non-sensitized bacteria.

By HOMER F. SWIFT, M.D. and RALPH A. KINSELLA, M.D.

[*From the Medical Clinic, Presbyterian Hospital, Columbia University, New York.*]

In a previous communication[1] we reported that the serum of rabbits which had been injected with sensitized vaccine or living cultures of *Streptococcus viridans*, did not contain agglutinins, complement fixing antibodies or protective antibodies. Later similar results were obtained with sensitized vaccines of pneumococci, with the exception that the serum of animals inoculated with sensitized living pneumococci showed a rapid production of these antibodies. We then attempted to determine whether there was evidence of active immunity even though no antibodies could be demonstrated in the serum. It has been found impossible to immunize mice with green streptococci. Rats may be immunized with green streptococci, but the virulence of these organisms is so low that it is impossible to compare the results of immunity

[1] Homer F. Swift and Ralph A. Kinsella, PROC. SOC. EXP. BIOL. AND MED., 1915–16, XIII, 103.

with the different forms of vaccines. For comparative study pneumococci are more satisfactory because of the high virulence of these organisms. Throughout the work type I pneumococcus has been used.

Four types of vaccine have been studied, (*A*) plain stock vaccine, killed at 56°; (*B*) sensitized stock vaccine, killed at 56°; (*C*) freshly prepared sensitized vaccine, killed at 56°; and (*D*) an alcohol precipitate of sensitized vaccine similar to that used by Gay in the preparation of typhoid vaccine.

Immunization was carried out by intraperitoneal injection of increasing quantities at three or four day intervals. Immunity has been studied in mice, guinea pigs and rats.

Mice immunized with three injections and tested from seven to eleven days after the last immunizing dose, showed immunity from all types of vaccines. The mice immunized with vaccine *D* were uniformly less immune than those with the other vaccines. There were only slight differences in the animals immunized with plain and sensitized vaccine, and in different experiments the results varied so that neither vaccine can be said to be better.

Guinea pigs received five immunizing injections in two and a half weeks. In series *A* there was moderate active immunity; series *B* very slight; and in series *C* none. The serum from two animals in each series was tested for antibodies and only in the serum of series *A* were agglutinins demonstrated.

Rats were found to be the most satisfactory animals for comparative studies. It was found that in six to ten days after the last immunizing dose, there was a higher degree of immunity in the plain vaccine series, but that this fell off rapidly. On the contrary, in the series immunized with freshly sensitized vaccine, the immunity though present was less marked early but increased after twelve to sixteen days. There was no parallelism between the degree of active immunity and the amount of agglutinin and bacteriotropin in the serum of the immune rats. Agglutinin was demonstrated only in the serum of series *A* rats. Bacteriotropins were much stronger in the series *A* rats than in the serum of series *C*. It is, therefore, evident that animals may possess a high degree of active immunity and still show practically no antibodies in their serum.

It is suggested that the immunity is due in part to a tissue immunity and not due entirely to antibodies circulating in the blood serum.

72 (1250)

The influence upon the Ehrlich sarcoma of stimulation or depression of the oxidative processes in mice.

By F. D. BULLOCK, M.D. (*by invitation*).

[*From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director.*]

As far as the reports in the literature show, no one has investigated the effect of drugs which increase or decrease the oxidative processes of the organism upon the transplanted tumors of mice.

Through the courtesy of Dr. A. S. Loevenhart, both iodozobenzoic and iodoxybenzoic acids, which are oxidative stimulants, were obtained. These acids were converted into their sodium salts and injected into mice both before and after inoculation with tumor. In one group of animals, sodium iodozobenzoate in doses of 0.015 gm. was injected subcutaneously every day for six days previous to inoculation with tumor, while in a second group the same drug in similar dose was injected for eight days after inoculation. A third group received six injections of sodium iodoxybenzoate in dosage of 0.002 gm. previous to inoculation, while the animals of a fourth group received eight injections of similar dosage after inoculation. A fifth group of control animals remained untreated.

As an oxidative depressant sodium cyanid was used. One series of animals received seven injections of 0.00062 gm. previous to inoculation, while another received seven injections of similar dosage after inoculation; a third untreated group served as controls.

All the animals were inoculated by needle with 0.003 gm. of the Ehrlich mouse sarcoma.

Neither of these two procedures influenced the percentage of takes or the rate of growth.

[1] Loevenhart, A. S., Harvey Lectures, 1914–15, X, 280.

73 (1251)

Influence of age on the permanence of subcutaneous autografts of the spleen in rabbits.

By DAVID MARINE and O. T. MANLEY.

[*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, Ohio.*]

We have removed the spleen and auto transplanted in 49 rabbits, varying in age from 26 days to over two years.

The method consists of introducing a small fragment of spleen, roughly 2–3 mm. in diameter, beneath the subcutaneous fascia of the abdomen, through a skin incision which is then closed by suture. These transplants have been subjected to direct examination at approximately monthly intervals to check their taking, growth or absorption. All have taken, except for three instances and these failures were due to infection. The most striking observation was the rapid growth of the grafts in the young rabbits from one to three months old and the lack of growth in the one- and two-year-old rabbits, some of which have undergone complete absorption in three months, as shown by histological examination. On the other hand, none of the transplants in rabbits less than five months old have shown any tendency to undergo absorption and histological examination shows regeneration of the major splenic elements into normal looking, encapsulated, highly vascular little spleens.

We have not been able to complete the series, with rabbits of known ages, between the period of sexual maturity (fifth month) and one year. The marked growth and activity of the transplants in young rabbits as compared with the lack of growth and tendency to absorption in old rabbits may be a part of the normal growth of the animal. In favor of this view is the fact that further growth of the transplants has not been observed after adolescence. There are no reports of differences in the systemic effects of splenectomy relative to age, although there is some evidence from histological studies that a blood-forming function is present in early life and absent in adults. It is suggested that the age differences noted in the growth and activity of spleen auto-

grafts in addition to their probable relation to the normal growth of the animal as a whole may also be related to the loss of one of the spleen's functions in early life, through that function being assumed by another tissue.

The ease with which the spleen of young rabbits can be autotransplanted into the subcutaneous tissues might be utilized in the study of its reactions or in chemical examinations, where multiple or control spleens in accessible locations are needed.

74 (1252)

Chemical evidence for the presence of glycogen-like polysaccharide in the liver blood of diabetic animals.

By J. J. R. MACLEOD.

[*From the Western Reserve University, Cleveland, Ohio.*]

Histological evidence for the presence in the hepatic capillaries of diabetic animals of material which stains by Best's carmine method in the same manner as the glycogen present in the liver cells (Huber and Macleod) has led us to investigate whether a polysaccharide could be separated by chemical means from the blood of the vena cava. In the first experiments of this nature (with G. E. Simpson), the blood of the vena cava was collected in excess of alcohol and the resulting precipitate treated with KOH in the usual manner. From the hydrolysis mixture a small amount of alcohol-precipitable material was secured, which after purification gave a violet color with iodine and exhibited reducing properties on hydrolysis. This material was sometimes isolated from the blood of normal animals (etherized), as well as from that of diabetic animals. In all cases, however, the yields were very small and uncertain, so that I have recently changed the method of isolation. Instead of precipitating with alcohol as the first step, the blood is now received directly into saturated alkali. On account of the high cost of KOH, a series of preliminary experiments were performed to see whether, after hydrolysis of the protein and heating with NaOH, a solution would be obtained from which the glycogen could be quantitatively precipitated by alcohol.

After heating solutions consisting of equal volumes of a relatively pure glycogen solution and of saturated NaOH (purified by alcohol) on the boiling water bath for periods exceeding three hours, it was found that the glycogen could not be precipitated by the addition of an excess of alcohol, unless the solution was almost neutralized with HCl and therefore contained an excess of sodium chloride. In the above manner it was found that a very high percentage all of added glycogen could be recovered.

When blood that had been removed from the carotid artery or portal vein of normal animals was treated in the above manner, a small amount of highly colored precipitate separated out on the addition of alcohol, but by redissolving this in boiling water after thorough washing with alcohol, a solution was obtained which gave only a faint opalescence on the addition of excess of alcohol and of NaCl. In one or two instances it was possible to collect this faint precipitate on a filter paper, but it was found to give no coloration with iodine, and after hydrolysis to yield a solution having no reducing properties. It probably consisted largely of inorganic material. On the other hand, when the blood was taken from the vena cava, opposite the point of entrance of the hepatic veins, of sugar-fed dogs, rendered hyperglycemic either by the injection of adrenin or by splanchnic stimulation, or from the carotid artery of piqûre rabbits, a much larger amount of precipitate was thrown down by alcohol (and NaCl), and after repeated reprecipitation, and in one case after a second digestion of the precipitate with half saturated NaOH, a pure white precipitate, amounting in one case to 0.0447 gm. after drying, was secured. This precipitate still contained a considerable quantity of alkali, etc., but when it was dissolved in water and the resulting solution neutralized, a deep red violet color was obtained with iodine (disappearing on heating and returning on cooling), and the solution rotated polarized light to the right. On hydrolysis, a solution was obtained that reduced Fehling's solution strongly, and gave abundant osazone crystals, which, however, even after recrystallization were mixed with so much amorphous material as to make melting-point determinations useless. Larger quantities of this polysaccharide are being collected for a more exact study of its chemical relationships.

75 (1253)

Soluble substance of pneumococcus origin in the blood and urine during lobar pneumonia.

By A. R. DOCHEZ and O. T. AVERY.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York.*]

A specifically reacting substance of pneumococcus origin has been demonstrated in the filtrates of young cultures of pneumococcus, and also in the blood serum and urine of patients during the course of lobar pneumonia. In bacteria-free filtrates of broth cultures of pneumococcus there is present a soluble material which gives a specific precipitin reaction with antipneumococcus serum. The filtrates from the different types of pneumococcus show the same specificity of reaction with immune serum as do the original cultures from which they are derived. The soluble substance present in the filtrates is undoubtedly of bacterial origin. That it is a product of the life processes of the pneumococcus and not due to its disintegration is shown by the fact that it is present in considerable amounts during the early stage of development of the culture when the organisms are growing at their maximum rate and undergoing little or no cell destruction as indicated by their growth curve.

The demonstration of the formation of this soluble substance in cultures of pneumococcus growing actively in vitro suggested the probability of its production in experimentally infected animals and in human beings suffering from lobar pneumonia. If rabbits are infected intraperitoneally with pneumococcus a substance specifically precipitable with antipneumococcus serum can be demonstrated in their blood serum, freed from bacteria by filtration, from within two to six hours following the time of infection. This soluble specific substance is also present in the blood serum during the course of lobar pneumonia in man and gives a precipitin reaction with antipneumococcus serum corresponding in type to the organism with which the individual is infected. This soluble precipitable substance is less frequently present in demonstrable quantities in human serum than in the serum of experimentally

infected animals. However, it has been found both when pneumococci were present in the circulating blood and when, by blood culture, organisms were absent. Complement fixation, as well as the precipitin reaction, may be used for the demonstration of this substance in serum.

The fact that considerable quantities of soluble material formed by the pneumococcus are present in the circulating blood in infection suggested the likelihood of its excretion in demonstrable form in the urine. Study of the urine by precipitin reactions in animals experimentally infected with pneumococcus and in a large series of individuals with lobar pneumonia showed a specifically precipitable substance to be present in almost every instance during some stage of the disease. A positive reaction has been found as early as twelve hours after the initial chill, and has been demonstrated in one instance five weeks after defervescence of fever. The reaction in different urines may vary from a faint cloud to a heavy precipitate. In certain instances the reaction may be negative when whole urine is used but become positive by chemical concentration of the substance in the same urine.

A study of the chemical nature, toxicity, antigenic properties and fate of the soluble substance in normal and infected animals is still in progress. The following facts, however, have already been ascertained concerning its chemical nature. The specific substance is not destroyed by boiling. It is readily soluble in water, precipitable in acetone, alcohol and ether. It is precipitated by colloidal iron and does not dialyze through parchment. Its immunological reactions are unaffected by proteolytic digestion with trypsin and the substance is not split by urease. The determination of total nitrogen and nitrogen partition on the active substance obtained by repeated precipitation with acetone and alcohol, shows this substance to be of protein nature or to be associated with protein.

76 (1254)

Cellular and humoral factors in anaphylaxis and immunity.

By W. H. MANWARING, ARTHUR R. MEINARD and YOSHIO KUSAMA.

[*From the Department of Bacteriology and Experimental Pathology, Leland Stanford Jr. University.*]

Our analyses of the anaphylactic and immune reactions by means of perfusion experiments with isolated rabbit and guinea pig tissues have shown that the hypersensitive and immune, humoral and cellular factors may coexist in the bodies of anaphylactic and immune animals in the following combinations:

(*a*) *Cellular anaphylaxis* and *apparently normal blood condition*. This is illustrated by the lungs of four-week anaphylactic guinea pigs. Tested in the presence of normal blood, these tissues give a typical anaphylactic reaction. The blood perfused through normal lungs, produces no recognizable response. We refer, of course, only to the immediate anaphylactic response; the slow production of toxic phenomena being beyond the scope of the present analyses.

(*b*) *Cellular anaphylaxis* and *humoral anaphylaxis*. This is illustrated by the lungs of fourteen-day anaphylactic guinea pigs. Tested in the presence of normal blood, these tissues are markedly hypersensitive. The blood, perfused through normal lungs, produces a typical anaphylactic response.

(*c*) *Cellular anaphylaxis* and *humoral immunity*. This seemingly paradoxical phenomenon is illustrated by the lungs of immunized guinea pigs. Tested in the presence of normal blood, these tissues are markedly hypersensitive. The blood, perfused through anaphylatic lungs, prevents the anaphylactic reaction.

(*d*) *Cellular immunity* and *humoral anaphylaxis*. This second seeming paradox is illustrated by the hearts of anaphylactic rabbits. Tested in the presence of normal blood, these tissues are distinctly resistant. The blood, perfused through normal hearts, produces a typical anaphylactic response.

(*e*) *Cellular immunity* and *humoral immunity*. This is illustrated by the hearts of immune rabbits. These tissues are dis-

tinctly resistant. The blood prevents the reaction if mixed with anaphylactic blood.

(*f*) *Humoral anaphylaxis* and *humoral immunity*. This third seeming paradox is illustrated by the blood of partially immunized rabbits, which contains a thermo-labile anaphylactic substance, partially inhibited by a thermo-stable antitoxin.

77 (1255)

Absorption of foreign protein by the anaphylactic lungs.

By W. H. MANWARING and HAROLD E. CROWE.

[*From the Department of Bacteriology and Experimental Pathology, Leland Stanford Jr. University.*]

If the lungs of an anaphylactic guinea pig are repeatedly perfused with dilute foreign protein, either in Locke's solution or in 50 per cent. normal blood, the lungs are thrown into a typical anaphylactic response.

Quantitative titrations of the perfusion fluid, by means of a specific precipitating serum, show no recognizable changes in the amount of protein as a result of the repeated passages through the lungs.

The titrations therefore furnish no support, either for the sessile receptor hypothesis of Ehrlich, or for the protein-destruction theory of Vaughan.

SCIENTIFIC PROCEEDINGS

Abstracts of Communications.

Eighty-third Meeting.

University and Bellevue Hospital Medical College, April 18, 1917.
President Gies in the chair.

78 (1256)

Quantitative experiments demonstrating the mechanism of the inhibition of growth.

By Jacques Loeb.

[*From the Laboratories of the Rockefeller Institute for Medical Research.*]

The problem of regeneration can also be stated in a negative way, namely: Why do tissues and dormant anlagen of organs not grow in an intact organism, while we know that they grow when the buds or tissues are isolated? The laws of inhibition were studied by a quantitative method.

No notch of a leaf of *Bryophyllum* will grow while the leaf is a part of the whole plant but the notches will grow when the leaf is isolated. When a leaf is subdivided into as many pieces as there are notches, each notch will give rise to a shoot; but if the leaf remains intact only few will grow out. The writer concluded that this again is a phenomenon of inhibition.

This inhibition he had explained in former papers[1] as being due to the fact that the inhibiting organ takes away the material required for the growth of the inhibited organ. If this were the case, we should expect that the total mass of shoots produced by a leaf in a certain time is approximately the same no matter whether the leaf produces few or numerous shoots. This is true

[1] Loeb, J., *Bot. Gaz.*, 1915, LX, 249; 1916, LXII, 293; 1917, LXIII, 25. "The Organism as a Whole," New York, 1916, p. 153.

to a surprising degree of exactness and the following numerical relations were established:

1. If a pair of sister leaves (of equal size) are isolated both will produce under equal conditions and in equal time approximately the same mass of shoots, although the number of shoots may differ considerably in the two sets of leaves.

2. If the mass of one of the two sets of sister leaves is diminished (by cutting out pieces of the leaves), the mass of shoots produced in the two sets is in proportion to the masses of the two sets of leaves.

3. It had been shown by previous experiments that if a piece of stem is attached to a leaf the growth of the notches of the leaf is retarded. It has been shown by the new experiments that the mass of shoots of leaves attached to a piece of stem is smaller when the mass of the stem is larger and that it also varies directly with the size of the leaf. These quantitative data furnish the basis for a chemical theory of regeneration.

79 (1257)

A new method of obtaining samples of the respiratory gases in animals. A demonstration.

By A. L. MEYER.

[*From the Department of Physiology and Pharmacology, Rockefeller Institute for Medical Research.*]

I wish to demonstrate a new method of obtaining a sample of air from the lungs, bronchi and trachea of the dog. Chloretone, dissolved in olive oil, is given intraperitoneally. The animal is tracheotomized and a T-shaped glass cannula is introduced. A Meltzer pleural cannula (new form) is placed in each pleural cavity. The intrathoracic negative pressure is then restored and the pleural spaces tested for air-tightness. It should be possible to maintain the negative pressure indefinitely. If any change occurs, it must be in the direction of an increase owing to the absorption of gases through the pleuræ.

Both pleural cannulæ are now connected with a source of air

pressure. A small-sized rubber bag is then attached to the horizontal portion of the tracheal cannula. The bag is thoroughly exhausted. The cocks of the pleural cannulæ are now opened. *At the end of an expiration*, the upright portion of the tracheal cannula is quickly clamped, the air pressure is turned on and the bag opened. A mercury valve is provided so that the desired pressure cannot be exceeded. The lungs collapse. Their contents are forced into the trachea and rubber bag. A portion of the air remains in the bronchi and trachea. The sample therefore approximates the *total air*.

It has been found that when no precaution is observed to maintain the body temperature, very uniform percentages of carbon dioxid may be obtained. Five experiments have been made thus far. In each experiment the carbon dioxid exhibited uniformity, either immediately or after a preliminary period of fluctuation. The periods of constancy ranged from one and a half to three and a half hours. In three experiments in each of which 8 determinations were made, the maximum deviation from the average was ± 3.0–3.5 per cent.; in one experiment, in which 7 determinations were made, the maximum deviation from the average was ± 2.4 per cent.; in another experiment, in which 5 determinations were made, the maximum minus deviation was 0.5 per cent. and the maximum plus deviation 1.4 per cent.

80 (1258)

On the compensation of the ocular and equilibrium disturbances which follow unilateral removal of the otic labyrinth.

By **A. L. PRINCE** (by invitation).

[*From the Physiological Laboratory, Yale Medical School.*]

It has been shown[1] that the disturbances of function which follow unilateral destruction of the otic labyrinth are of short duration in higher mammals, the torsion of the head being the only persistent symptom. In very young animals, the conditions

[1] Wilson and Pike, *Phil. Trans. Royal Society*, London, 1912, Series B, CCIII, p. 127.

may be somewhat different.[1] The symptoms in lower forms persist for a longer time. The question arises as to the mechanism of the compensation for the injury in higher mammals.

The experiments reported in this paper show that the mechanism for compensation involves the cerebral hemispheres.

The experiments were conducted with young cats except in one case in which a fully grown animal was used. The otic labyrinth was removed under asepsis according to the method described by Wilson and Pike.[2] At various periods following the labyrinthine operation, at a time when all ocular and equilibrium disturbances had disappeared, the animals were decerebrated by section of the brain stem just anterior to the corpora quadrigemina.

In view of their uniformity a general description of the results will suffice.

I. *The effect of complete decerebration on the position of the eyes in animals which have fully recovered from the ocular symptoms resulting from unilateral removal of the labyrinth.*

In all experiments the ocular movements were absolutely normal at the end of three days following removal of the labyrinth. At the end of 3, 11, 12, 16, and 24 days after the labyrinthine operation the animals were decerebrated. This operation was followed by an intense deviation of the eyes to the side of the labyrinthine lesion. The degree of deviation of the eye on the side of the intact labyrinth was somewhat less marked. The deviation of the eyes, as far as could be determined from the nature of the experiments, is permanent. That pure deviation and not nystagmus recurs on decerebration is readily explained by the observations of Wilson and Pike.[2] These author found that the quick return phase is dependent on the integrity of nervous paths in the region of the cerebrum.

II. *The recurrence of disturbances of equilibrium following complete decerebration in animals which have fully recovered from the symptoms resulting from unilateral removal of the labyrinth.*

The animals were untied and observed for several hours after

[1] Prince, *American Journal of Physiology*, 1917, XLII, p. 308.

[2] Wilson and Pike, XVIIth International Congress of Medicine, London, 1913, Section XVI, Otology, p. 563; *Arch. Int. Med.*, 1915, XV, 31; PROC. SOC. EXP. BIOL. AND MED., 1917, XIV, p. 75.

decerebration. Before decerebration the animals presented no disturbances of equilibrium. For several hours after this operation the animals presented all the bodily reactions which appear on the first day following unilateral removal of the labyrinth. The protocols of one of these experiments follow:

Kitten, weight 1450 g.

Feb. 15, 1917. Right otic labyrinth removed under asepsis. Operation followed by nystagmus and typical disturbances of equilibrium, violent in character.

March 3 (16 days following labyrinthine operation).

2.40 P. M. Decerebration under ether. Section just anterior to corpora quadrigemina.

2.55 to 6.30 P. M. Marked and permanent deviation of eyes to right (side of labyrinthine lesion). Animal tied to turntable with the head fixed in the vertex upward position. When the direction of rotation is toward the side of the labyrinthine lesion, the deviation of the eyes is intensified on cessation of rotation. The eyes then return slowly to the initial position of deviation. When the direction of rotation is toward the intact labyrinth, deviation entirely disappears on cessation of rotation; the eyes then return slowly to the initial position of deviation. Animal removed from rotation board. When removed from the board, the animal lay on its right side with fore limbs extended. The neck was bent to the side of the labyrinthine lesion. Marked increase in torsion of the head, occiput pointing to the right. When disturbed by pinching the tail, the animal made violent attempts to turn on its dorso-ventral axis toward the side of the labyrinthine lesion. The forelimbs participated actively in the attempts to turn to the right. When the animal was placed on its back or on its left side, it immediately rolled to the right and came to rest in the position described.

6.30 P. M. Observations made at frequent intervals since last note without noticeable changes in the reactions.

CONCLUSIONS.

The disappearance of the ocular and the equilibrium disturbances following unilateral removal of the labyrinth is attributed to the activity of a compensatory mechanism.

As the labyrinthine symptoms recur after complete decerebration, the nervous paths concerned in the process of compensation may be roughly localized in the cerebrum above the level of the corpora quadrigemina.

81 (1259)

Diuretic effects of the caffeine group.

By GEORGE B. WALLACE and E. J. PELLINI.

[*From the Department of Pharmacology, University and Bellevue Hospital Medical College, New York.*]

The knowledge concerning the diuretic action of caffeine and theobromin has been obtained almost entirely through work on rabbits. It is from this work that the many explanations of how these drugs act in producing diuresis have arisen. The dog was early recognized as being somewhat refractory or uncertain in his response, and consequently but little work has been done on this animal. It has seemed to us however that the dog's urinary function is much more comparable to that of man than is the rabbits, and we have accordingly carried out a series of diuretic experiments using dogs as the experimental animals.

The dogs used were placed on a fixed diet, with a fixed daily intake of water. We have collected and analyzed the urine in twenty-four periods in order to avoid the frequent and unexplained variations which occur in shorter periods. Drugs were withheld until a comparatively constant daily output of urine both in quantity and composition was obtained. Caffeine, theobromin or theobromin sodio-salicylate was then given in dosage varying from 0.05 to 0.2 g. three times a day, for periods of from one to five or more days. They were given with the food and in capsules.

The results may be summarized as follows: With none of these drugs, in the dosage employed have we seen any appreciable increase in the urine output. On the contrary there has been almost invariably a decrease. When the drug is given for one day only, the decrease may occur on that day or the one following. When given for several days the decrease usually continues during the entire period, although in some instances the urine output

may return to the normal level on the fourth or fifth day. In general the larger doses have produced the more marked decrease. The total nitrogen, urea and sodium chloride decrease fairly proportionately to the water. During the period of drug action, that is when the output of urine is definitely decreased, the renal function seems unimpaired, since water, urea and sodium chloride added to the regular diet, are excreted by the kidney in a normal fashion. Phenolsulphonephthalin also is excreted in the same percentage as during the control period. We have analyzed the blood during the control period and during the period of drug action. In this latter period, when the urine output is below the normal, the non-protein nitrogen, urea nitrogen and sodium chloride in the blood are decreased. This does not appear to be due to an increase in water in the blood since the percentage of water is also slightly decreased.

From these results we conclude that there may be two factors which determine the diuretic action of these drugs in the dog and probably in man, one an action on the kidney, similar to that in the rabbit tending to cause diuresis, a second and determining one on the tissues in general, as a result of which water and excretory products are held back by the tissues.

82 (1260)

Cholesterinized alcoholic extracts versus acetone insoluble fraction of pure tissue lipoids as antigen for Wassermann reaction.

By J. BRONFENBRENNER and M. J. SCHLESINGER.

[*From the Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Pa.*]

In the last few years a number of workers in this country have reported favorably on the use of cholesterinized antigens for Wassermann reaction. According to some reports, these so-called reinforced antigens gave even more satisfactory results than the acetone-insoluble fraction of tissue lipoids advocated by Noguchi.[1]

[1] Noguchi and Bronfenbrenner, *Jour. of Experimental Medicine*, 1911, Vol. XIII, No. 1, p. 43.

In studying the rôle of cholesterin in the preparation of antigen we came to the following conclusions:

The apparent superiority of reinforced antigens is not due to the heightened specificity of these antigens, but to a heightened anticomplementary power of the same.

In addition, cholesterol retards and, if used in sufficient connection, greatly protects the blood cells from the action of hemolytic agents, thus further increasing the tendency towards a higher percentage of positive reactions.

That this influence of cholesterol is not specific is evidenced by the fact that more recently different authors noted the occurrence of nonspecific fixations obtained with reinforced antigen in a high percentage of normal cases.

The erroneous procedure recommended by some of the recent texts of using $\frac{1}{2}$ to $\frac{1}{3}$ of anticomplementary dose of reinforced antigen in the test may increase the occurrence of nonspecific fixations up to 40 per cent. of normal cases, as reported by some of the investigators.

On the other hand we found that removing all the cholesterol from the tissue extracts actually improves them for the use in the complement fixation test.

By redissolving and reprecipitating the acetone insoluble fraction of tissue lipoids one does not diminish its antigenic value, while one very markedly decreases its anticomplementary power.

The antigen prepared in this way can be used in the amount 10 or even 20 times smaller than the dose fixing 1 H E unit[1] of complement, and yet even such minute amounts of antigen may contain considerably more than 10 antigenic units.

In general we can not but discourage the use of cholesterinized antigens in favor of reprecipitated acetone insoluble fraction of tissue lipoids free from cholesterol.

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[1] Bronfenbrenner and Schlesinger, *Am. Jour. of Syphilis*, April, 1917.

83 (1261)

The effect of temperature on the rate of complement fixation.

By J. BRONFENBRENNER and M. J. SCHLESINGER.

[*From the Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Pa.*]

Suitable temperature is one of the most important prerequisites to the proper progress of biological reactions. Since the complement fixation reaction is used so widely at present and the relation between the temperature and rate of fixation has not been studied systematically thus far, we thought it advisable to study this question. This was especially necessary, since many authors have varied the technique of the routine complement fixation test in this respect. The practical points brought out in this study are as follows:

1. If a rapid fixation of complement is desired we found that a temperature of 37° C. is the best. For diagnosis one-half-hour incubation at this temperature is the most efficient. We found, however, that, if sufficient antibody is present in the serum (3–5 units or more), fixation of two units of complement already takes place within the first five minutes, provided the amount of antigen used contains several antigenic units. We find it possible to use this procedure for presumptive elimination of strongly positive sera from a large series of cases. One places in a tube 0.05 c.c. of the patient's serum, adds the proper amount of antigen and salt solution and incubates at 37° C. in the water bath for five minutes and then adds sensitized cells to test for free complement.[1]

2. If the time element is not so important, but complete fixation of the complement is desired, then we find that incubation in the ice box for 8–10 hours is best. These fixations on ice, however, may not be specific, for the reaction of fixation is so complete under these conditions that even traces of secondary circulating antigens and their corresponding antibodies may cause

[1] One must, of course, test 0.025 c.c. of the serum for complement at the same time, to ascertain that there are at least two units of complement originally present in the amount of serum used in the test.

fixation of complement.[1] The ice-box fixation can therefore be used only as a presumptive test to eliminate the negative cases.[2]

3. As for fixation of complement at temperatures below the freezing point we find that such a procedure produces undesirable changes in the reagents, especially in the antigen and is therefore unsuitable for the test.

84 (1262)

A study of the acid-base equilibrium of the blood in acute bichloride intoxications.[3]

By WM. DEB. MACNIDER.

[*From the Laboratory of Pharmacology of the University of North Carolina.*]

Thirty-two dogs have been used in the initial series of experiments. The animals were kept in metabolism cages, given 500 c.c. of water by stomach tube and fed on bread with a small amount of meat. The urine was examined qualitatively for albumin, acetone and glucose. The hydrogen ion (p. H.) concentration of the blood was determined by the method of Levy, Rowntree and Marriott, and the alkali reserve of the blood (R. p. H.) and the tension of alveolar-air carbon dioxide by the methods of Marriott. The phenolsulphonephthalein test was conducted according to the method of Rowntree and Geraghty.

The urine during two days of observation prior to the administration of the bichloride was normal. The hydrogen-ion content of the blood varied between 7.4 to 7.5, the reserve alkali between 8.05 to 8.1, while the tension of carbon dioxide in alveolar air has shown a variation between 40 to 45 mm. The total output of phthalein in a two-hour period has varied from 74 to 91 per cent.

The animals were starved for twenty-four hours prior to giving the bichloride. On the days of the experiments the animals were given 0.25 c.c. of a 4 per cent. solution of morphine sulphate

[1] Bronfenbrenner and Schlesinger, PROC. SOC. EXP. BIOL. AND MED., 1916, XVI, p. 37.

[2] Bronfenbrenner and Schlesinger, *Am. Jour. of Syphilis*, April, 1917.

[3] The expense of the investigation has been aided by a grant from the Rockefeller Institute for Medical Research.

subcutaneously. After the initial emesis the animals became drowsy and during this period 15 mg. per kilogram of bichloride of mercury was given by stomach tube. With four exceptions no vomiting occurred from the local irritant effect of the mercury.

Within twenty-four hours all of the animals developed a severe gastro-enteritis. The stools were frequent and bloody. The severity of the enteritis has varied in different animals. There is no definite relation between the severity of the gastroenteritis and the delayed toxic effect from the poison. The variation in the toxic effect of bichloride in the different experiments permits the following classification of the animals.

Group 1.—Six animals. An intense gastroenteritis. The animals are in much pain, cold, tongue bluish, unable to walk. Apparently in collapse. All of the animals in this group which is characterized by the early and intense gastroenteritis have died within forty-eight hours following the bichloride.

The urine in this group is reduced in amount. The lowest output in twenty-four hours has been 91 c.c. With one exception, the urine of these animals has been free from albumin, glucose and acetone. The urine of one of the animals contained less than 0.5 gm. of albumin per liter (Esbach). In this group of animals which apparently die from the shock associated with the intense local action of the bichloride, the hydrogen-ion content of the blood has not gone higher than 7.35. The reserve alkali of the blood and the tension of alveolar air carbon dioxide have not varied from the normal. The phthalein output has been uniformly slightly reduced.

Group 2.—Nine animals. All of the animals have developed a severe gastroenteritis. During the subsidence of the enteritis or several days after the symptoms of the enteritis had disappeared, the animals have shown an increase in the hydrogen ion content of the blood which in one animal went as high as 7.3. The alkali reserve of the blood has shown a depletion. In none of the animals have the determinations been below 7.9. The tension of carbon dioxide has not gone below 35 mm. In this group of animals the phthalein output has shown a greater reduction than in Group 1. The total output has varied between 51 to 63 per cent. The urine has contained as much as 1.5 gm. of albumin per liter. No acetone or glucose.

Following these indications of a mild acid intoxication and an associated kidney injury, the acid-base equilibrium of the blood has gradually returned to the normal, the output of phthalein has increased, the albumin has disappeared or has been reduced to a trace and all of the animals have recovered.

Group 3.—Seventeen animals. Following the subsidence of the mercury enteritis, and in the case of two of the animals, as late as six days after these symptoms had disappeared, the animals have either very rapidly (within eight hours), or gradually, developed a severe acid intoxication and died. The highest hydrogen ion concentration of the blood in this group has been 7.1. The reserve alkali of the blood in this animal was reduced to 7.7, while the tension of alveolar air carbon dioxide was 18 mm. The animal died in air hunger.

With the development of an acid intoxication the output of urine is greatly reduced or the animal is rendered anuric. The phthalein output shows a rapid reduction. In the animal above referred to the output of phthalein was only 10 per cent. The urine in this group of animals has contained as high as 2.75 gm. of albumin per liter and in all of the animals the urine has contained both glucose and acetone. Casts have not been numerous.

The kidneys of those animals which succumb during the height of the gastro-enteritis from bichloride are uniformly and severely congested. The kidneys appear cyanotic. The epithelium shows but slight evidence of damage. The cells of the ascending limb of Henle's loop show a small amount of fat.

The kidneys of those animals which die after the subsidence of the enteritis and during a period of acid intoxication have a pale, anemic appearance and show a clear-cut fatty zone at the cortico-medullary junction. The tubular epithelium, and especially that of the convoluted tubules, is severely swollen, vacuolated and rapidly becoming necrotic. The cells of the ascending limb of Henle's loop show a severe fatty infiltration.

CONCLUSIONS.

1. The acute kidney injury which develops late in poisoning by bichloride of mercury does not show any dependence in the frequency with which it occurs with the severity of the mercury enteritis.

2. The delayed kidney injury in acute bichloride intoxications has been constantly associated with the development of an acid intoxication. The severity of the damage to the cells of the convoluted tubules and the extent of fatty infiltration in the cells of the ascending limb of Henle's loop show a correlation with the degree of acid intoxication.

85 (1263)

Relation of the spinal cord to the spontaneous liberation of epinephrin.

By G. N. STEWART and J. M. ROGOFF.

[*From the H. K. Cushing Laboratory of Experimental Medicine, School of Medicine, Western Reserve University, Cleveland.*]

1. In acute experiments on cats, anesthetized with urethane, section of the spinal cord in the cervical region caused no demonstrable diminution in the rate of liberation of epinephrin from the adrenals as tested by allowing the adrenal blood collected in a cava pocket to elicit eye-reactions (after preliminary excision of the superior cervical ganglion). Four such experiments were made. The cord section in one of these was between the third and fourth cervical vertebræ, in another opposite the body of the fourth cervical vertebrae, in a third just below the body of the fifth cervical vertebra, and in a fourth cat between the fifth and sixth cervical vertebræ. In the second of these animals the blood was collected from the adrenal vein before and after section of the cord. The epinephrin assay (by rabbit intestine segments) gave the same output of epinephrin per minute after as before the section. The blood flow from the adrenals was much slower after the section, but was correspondingly richer in epinephrin.

2. In two survival experiments the cord was cut in the cervical region (in one just above the body of the last cervical vertebra, in the other at the level of the body of that vertebra). In the first cat, the superior cervical ganglion had been previously excised. Two days after the cord section adrenal blood was tested by the cava pocket method and gave good eye reactions, indicating

a fairly good liberation of epinephrin (at least 0.0004 mgm. per minute). In the second experiment blood was drawn three days after the cord section and tested on rabbit intestine and uterus segments. Good concentrations of epinephrin were found (1 : 1,500,000 in the fourth adrenal sample, more than 1 : 2,500,000 and less than 1 : 1,500,000 in the second adrenal sample). Although the blood flow was small (0.3 gm. per minute for the second sample) a substantial liberation of epinephrin was demonstrated.

3. In one acute experiment, the spinal cord was cut between the fifth and sixth cervical vertebræ. The pupil reaction was not noticeably diminished. The cord was then severed between the fourth and fifth dorsal vertebræ. The pupil reaction could no longer be obtained. When the cord was now stimulated with induction shocks between the fifth and sixth dorsal vertebræ, and blood collected in the cava pocket during stimulation, good eye reactions were elicited on releasing the pocket.

4. In one survival experiment, the cord was cut between the fifth and sixth dorsal vertebræ. Three days afterwards the adrenal vein blood was tested by the cava pocket method but no eye reactions could be obtained. The pupil gave a good reaction with 0.2 c.c. of 1 : 500,000 adrenalin. On intestine segments negative results were obtained with adrenal blood, although a concentration of 1 : 60,000,000 adrenalin in indifferent blood caused a distinct effect. It was shown that the adrenal vein blood could not have contained 1 : 100 000,000, and that the discharge of epinephrin per minute could not have been at most 0.000003 mgm., that is, not one hundredth of the output to be expected in a normal cat under the experimental conditions. It was not demonstrated that any epinephrin was present.

5. In three of the cats with the cervical card transected (1 survival, and 2 acute experiments), the effect on the eye reactions of severing nerves containing the fibers concerned in the liberation of epinephrin (sympathetics and splanchnics in thorax, splanchnics in abdomen, and other nerves coming to semilunar ganglion, lumbar sympathetic chain) was studied. The eye reactions still obtainable from the adrenal blood after the cervical section were greatly weakened or abolished after the division of those nerves.

6. It seems to follow from these observations, that liberation

of epinephrin from the adrenals is still sustained after division of the cord in the cervical region at the levels mentioned, and that this liberation takes place through the splanchnic and other nerves known to be concerned when the spinal cord is still connected with the brain. The contrast between the epinephrin output when the cervical cord has been divided and when the dorsal cord has been divided at the levels mentioned is very great.

86 (1264)

Quantitative experiments on the liberation of epinephrin from the adrenals after section of their nerves, with special reference to the question whether epinephrin is indispensable for the organism.

By G. N. STEWART and J. M. ROGOFF.

[*From the H. K. Cushing Laboratory of Experimental Medicine, School of Medicine, Western Reserve University, Cleveland.*]

1. We showed in a previous paper[1] by the blood pressure and eye reactions that after section of the nerve supply of the adrenal no demonstrable liberation of epinephrin was present in cats as long as five weeks after the nerve section.

2. As it is easier to detect very small concentrations of epinephrin by the rabbit intestine and uterus segments, we have made a series of experiments (on 7 cats) in which these tests were used to supplement the eye reactions. In all the animals one adrenal was excised and the nerves of the other cut.

In a cat tested two weeks after the operation, it was shown that the adrenal blood serum could not have contained 1 : 300,000,000, or the blood 1 : 400,000,000, of epinephrin; and that the rate of liberation of epinephrin could not have been at most 0.000001 mgm. per minute for one adrenal. In another cat three weeks after the operation[2] the serum of the adrenal blood was proved to contain less than 1 : 400,000,000, and the blood less than 1 : 700,000,000 epinephrin. The output of epinephrin per

[1] *Journal of Pharmacology and Experimental Therapeutics*, 1916, VIII, 479.

[2] In this animal after the usual operation the left semilunar ganglion and the first ganglion of the lumbar sympathetic chain below the diaphragm were excised.

minute could not have been as much as 0.0000000 mgm. per minute, for one adrenal. The segments used for the tests in these experiments were extremely sensitive, and the limits of adrenalin concentrations which could be detected with certainty were carefully determined. The eye reactions were negative. In these two cats the rate of liberation of epinephrin, if any liberation whatever was going on, must have been several hundred times less than the rate in normal animals under the same experimental conditions.

It is scarcely necessary to point out that experiments yielding completely negative results indicating the absence of epinephrin with very sensitive test objects are much more important for the questions studied than experiments in which small amounts of epinephrin can still be detected. For it is impossible to be certain that when a little epinephrin is found some of the fibers concerned in the liberation may not have escaped section.

3. Since these animals had completely recovered from the operation and behaved in every way like normal animals, it must be concluded that the liberation of epinephrin from the adrenals is not indispensable for life or health, unless indeed the necessary quantity is, even in the adrenal vein blood, below the limits of detection by the methods used. It must be remembered that the epinephrin in the adrenal blood is diluted enormously (probably at least 100 times) in the right heart; so that in these cats the concentration in the arterial blood could not at most have reached 1 : 40 billions and 1 : 70 billions, respectively.

If the liberation of epinephrin is abolished by division in the dorsal cord of the path concerned in it, as our experiments on "Relation of the Spinal Cord to the Spontaneous Liberation of Epinephrin" indicate, this corroborates the conclusion that epinephrin is not indispensable. Numerous animals and men have long survived such lesions.

4. These experiments indicate that the entire liberation of epinephrin from the adrenals is controlled by nerves.

5. In a third cat (8 days after operation) the adrenal vein blood contained epinephrin but in concentration not exceeding 1 : 125,000,000. The output of epinephrin per minute was probably not more at most than one-hundredth of what might be expected in a normal animal.

6. In a cat 15 weeks after the operation it was doubtful if any epinephrin was present in the adrenal vein blood. In two others 15 weeks after operation eye reactions and segment tests showed the presence of a small amount of epinephrin, the rate of liberation being a mere fraction of the normal. The possibility of regeneration of fibers after this interval must be considered. In the seventh cat (tested two weeks after the operation) the eye reactions were negative. The segment tests revealed a small concentration of epinephrin in the adrenal blood (less than 1 : 30,000,000) corresponding to a rate of liberation of epinephrin per minute of at most one tenth of the normal.

ABSTRACTS OF THE COMMUNICATIONS, PACIFIC COAST BRANCH, SEVENTEENTH MEETING, SAN FRANCISCO, CALIFORNIA, APRIL 4, 1917.

87 (1265)

The function of the kidneys under strain in uranium nephritis and the relationship between anatomy and function under these conditions.

By C. K. WATANABE, J. R. OLIVER, and T. ADDIS.

[*From the Laboratory of the Medical Division of Stanford University Medical School.*]

Rabbits were injected subcutaneously with uranium nitrate in doses which varied from those which produced marked anatomical and functional changes to those which led to no certain effect.

A strain was placed on the urea-excreting function of the kidneys by the administration of urea by stomach tube.

While the kidneys were under the influence of this strain, the volume of urine, the rate of urea excretion, and the concentration of urea in the urine and blood were determined before and after the injection of uranium.

The most marked and constant functional change produced by the uranium was found to be a depression of the ratio between the rate of urea excretion and the concentration of urea in the blood. The degree of depression in this ratio was fairly closely parallel to the degree of anatomical damage, as judged from the extent and intensity of the necrosis or of the degenerative changes found in the terminal portion of the proximal convoluted tubule.

SCIENTIFIC PROCEEDINGS

Abstracts of Communications.

Eighty-fourth meeting.

Zoological Laboratory, Columbia University, May 16, 1917.

President Gies in the chair.

88 (1266)

The calcium and magnesium metabolism of the dog during inanition.

By **Maurice H. Givens** (by invitation).

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.*]

During starvation for sixty days a well-nourished female dog was reduced in body weight from 12 to 5.8 kg. At the end of the first seven days the daily urinary calcium had dropped to one-eleventh of the original excretion, that is from 43 to 4 mgm. CaO per day, a level from which there was hardly any deviation during the remaining days.

The urinary magnesium excretion dropped very gradually, so that on the last day of the fast it was about half of the initial value. It diminished with the decrease in the dog's weight and in proportion to the nitrogen eliminated. Presumably the dog's magnesium excretion was correlated with the catabolism of the muscular tissue.

The fecal excretion of lime during the starvation was greater than the urinary, while the magnesium in the feces, for the period, was less than that of the urine.

Studies on realimentation with diets low in calcium are in progress.

89 (1267)

Influence of certain electrolytes upon the course of the hydrolysis of soluble starch by malt amylase.

By H. C. SHERMAN and JENNIE A. WALKER.

[*From the Laboratory of Food Chemistry, Columbia University.*]

The rate of formation of reducing sugar (maltose) from soluble starch by purified malt amylase of diastatic power equivalent to about 1.600 on Lintner's scale, both in neutral solution containing no added electrolyte and with the addition of regulated amounts of hydrochloric or phosphoric acid or of primary potassium phosphate, was investigated from the beginning of the reaction to completion or until the hydrolysis is no longer measurable.

When the activating electrolyte was added in such amount as to give optimum or nearly optimum concentration of hydrogen ion, the action of the enzyme was increased not only in the earlier stages but throughout the entire range investigated. The greater the concentration of enzyme the less the apparent favorable effect of the added electrolyte.

The same optimum hydrogen ion concentration, $C_H 10^{-4.4}$ (P_H=4.4), was found to hold for each of the acid electrolytes tested and appears to hold throughout the course of the hydrolysis. (With neutralized starch substrate used in this laboratory the amount of acid or acid phosphate required for optimum activation is about half as much for one per cent. as for two per cent. starch.)

When more than the optimum amount of acid was added the hydrolysis proceeded at less than the optimum rate throughout; when less, the initial rate was better sustained. This difference was most pronounced in the case of hydrochloric acid: less with phosphoric acid: least in the case of acid phosphate ("buffer effect").

With initial concentrations of 1 per cent. soluble starch it was found that, throughout the first half of the hydrolysis, or up to a yield of half the theoretical amount of maltose, the rate of maltose formation from soluble starch was found to be proportional to the concentration of substrate (in the form of starch and dextrin) still remaining at any given time, at least in solutions containing avorable amounts of acid or acid phosphate.

When, in similar experiments, enzyme concentration is varied within limits suitable for such quantitative study, the rate of maltose formation is found to be directly proportional to the enzyme concentration, provided comparison is made at a point not beyond that corresponding to a yield of about half the theoretical amount of maltose. This indicates the range within which diastatic activities may be compared quantitatively.

In the action of malt amylase upon soluble starch, we find no distinct "region of linear relationship" in which the yield of reducing sugar is directly proportional to time.

Experiments with widely varied enzyme concentration show that there is no cessation of hydrolysis nor true equilibrium at a point corresponding to 80 per cent. of the theoretical yield of maltose as claimed by some previous investigators.

90 (1268)

The effect of coagulation of the pancreas in situ.

By J. AUER and I. S. KLEINER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

By injecting 10–15 c.c. of 85–95 per cent. alcohol, usually with 0.7 per cent. glacial acetic acid, into the main pancreatic duct of dogs, we coagulated at least 95 per cent. of this organ in successful experiments. The extent of this coagulation was determined by a careful inspection at the time of injection, by reoperation after a number of weeks, and by autopsy and microscopical examination. The external secretion of the gland was abolished in all experiments.

Our material is formed by 19 dogs, of which six lived four weeks and longer; one of the four dogs still living is in excellent condition 104 days after the operation. The blood and urine were examined at frequent intervals, daily when necessary. The dogs were fed a regular mixed diet composed of about 100 grams of cooked meat scraps, 4–500 grams of bread-meat broth mush, 50 grams ground bone and occasionally 10–60 grams of lard. Water was given freely.

Our results are briefly as follows: in spite of the fact that at least 95 per cent. of the pancreas was *immediately* killed by the alcohol-acid mixture, and that the dogs were fed on a diet rich in carbohydrate, yet the great majority of our animals showed no glycosuria or hyperglycemia. Occasionally a faint trace of sugar appeared in the urine. The blood sugar varied in general between 0.10 and 0.15 per cent.; in some nervous dogs, which required considerable handling, the blood sugar at times reached 0.20 per cent.

There were two striking exceptions to this general course; two dogs developed a severe diabetes immediately after the operation. In dog 28, which died after 28 days, the urinary sugar varied between 3 and 6 per cent., and the blood sugar from 0.27 to 0.40 per cent. This dog showed a gangrenous pancreatitis. The second dog, no. 32, showed a severe diabetes for seven days after the operation, with a glycosuria of 2–5 per cent. and hyperglycemia which reached 0.32 per cent. From the 9th day, the urine was sugar free, though the blood then showed 0.23 per cent. sugar. Within a week the glycemia had fallen to 0.12 per cent. This animal is still alive after 36 days; moreover, this dog showed a complete tolerance for 10 grams dextrose per kilo when fed this amount 21 days after the operation.

It is important to add that both dogs, but especially the fatal case, were subjected to considerable traumatism during the operation, due to technical difficulties.

The tolerance for 10 grams of dextrose per kilo when fed with the ordinary mixed diet, was good when tested in five of our dogs 21 to 90 days after the operation. One dog excreted no sugar whatsoever; three excreted 0.3–0.7 gram per kilo; the remaining dog excreted 1.5 grams per kilo.

The feces of all dogs examined for a longer period were large, voluminous and often greasy. Microscopical examination always showed enormous numbers of undigested muscle fibers, the cross striations being clearly visible as a rule. Starch granules varied in quantity, often large amounts were present, at other times the iodine test showed relatively few. Fat also varied considerably; after feeding 50 grams of lard fat drops were abundant; after smaller amounts often no fat drops were seen. Such variations

have also been noted by other observers after excluding the pancreatic juice from the gut.

None of our dogs showed polyuria; 580 c.c. was the largest recorded daily output. Acetone was seen only exceptionally.

All of the dogs showed an initially rapid and then slowly progressive loss of weight. Thus No. 5 lost 3.25 kilos in 12 days, but during the next 90 days lost only a little more than one kilo.

The experiments are being continued.

91 (1269)

Does a fatigue toxin exist?

By FREDERIC S. LEE and B. ARONOVITCH.

[*From the Department of Physiology, Columbia University, New York.*]

In 1904 Weichardt claimed to have found a specific substance, a fatigue toxin, as the chief agent in the production of fatigue. When present it was capable of forming in the tissues its own antidote, an antitoxin. A substance identical with fatigue toxin, named "kenotoxin," was obtained in vitro by treating proteins in various ways. However obtained, the substance, when injected into animals in small quantity, resulted in the production of antitoxin; when in larger quantity, it caused a great reduction in bodily temperature, slowing of respiration, sleep, and ultimately death.

In the present experiments animals, such as rabbits or cats, were fatigued by running in a revolving wheel. After fatigue was pronounced the animals were killed by decapitation, and the muscles of the hind legs were stimulated directly by the faradic current until they ceased to contract. Soon after death, in some cases within six to eight minutes, marked rigor was observed in the skeletal musculature. Immediately after the cessation of response to faradic stimulation the muscles of the hind legs were removed, cut to pieces and ground thoroughly with sand, and the muscle juice was squeezed out by a powerful press, all procedures being carried on with aseptic precautions. The juice was found markedly acid to litmus. When this juice was injected into the

peritoneal cavity of guinea pigs, usually in quantities as large as 10 c.c., either at the temperature of the room or after being warmed to bodily temperature, the animal became quiet; there was no constant effect on respiration, which sometimes increased in rate and sometimes decreased; and the bodily temperature began to fall at once. The fall continued during 30 minutes to 1 hour, the maximum so far observed being 1.6° C., after which there was a slower return toward the original temperature. These are the only immediate effects that have been observed. Occasionally the animal died on the following day. Precisely the same effects, including occasionally death on the next day, were obtained when the muscle juice of non-fatigued animals was used.

The working power of excised gastrocnemius muscles of the frog, when suspended in a bath of muscle juice prepared from fatigued and non-fatigued cats respectively, was studied. With the juice from fatigued muscles the duration of the working period of the gastrocnemii and the total amount of work performed were each diminished by about one half when compared with normal muscles not treated with juice. Practically the same quantatitive effect was observed when the gastrocnemii were treated with non-fatigued juice.

There can be no doubt that by the methods employed above, the muscles were thoroughly fatigued. The conclusion seems justified that no acutely toxic fatigue substance was produced. Weichardt's assumption of the existence of a specific fatigue toxin is therefore not sustained. It seems probable that Weichardt's animals, which were actually killed by his extreme methods of inducing fatigue, were put into a profoundly pathological condition in which the toxic component of the protein molecule was split off. There is no reason to believe that this occurs in the normal course of fatigue.

92 (1270)

The differences between arterial and venous oxygen contents in heart failure.

By CHRISTEN LUNDSGAARD (by invitation).

[*From the Hospital of the Rockefeller Institute for Medical Research, New York.*]

The paper is a report of an attempt to estimate the circulation by the difference in the oxygen content of arterial and venous blood.

1. *Arterial Blood.*—Being unable to draw samples from an artery, we take the venous blood, saturate it with air, and determine either its oxygen content or its hemoglobin content, which is proportional to the oxygen capacity. The oxygen content is determined by Van Slyke's method, previously described in the Proceedings of this Society. The hemoglobin is determined by a colorimetric method of Dr. W. W. Palmer.*

2. *Venous Blood.*—Ten c.c. of blood, drawn from the arm vein without any stasis, is deposited together with a little oxalate below mineral oil. A sample of 2 c.c. is transferred to the chamber of the Van Slyke apparatus without exposure to air, and the chemically bound oxygen determined.

RESULTS.

Twenty-three determinations have been done on 7 normal persons in rest and 27 on 13 resting patients.

1. *Normals.*—The amount of oxygen taken away has varied from 2.5 to 9 volumes per cent.

2. *Patients.*—In clinical compensated patients the difference between arterial and venous oxygen fell between 2.5 and 9 volumes per cent. In clinically decompensated, it was usually about 9 volumes per cent.; the highest found was 16. Three patients are followed under treatment.

The difference between arterial and venous oxygen appears, therefore, to be related to the degree of retardation in the circulation, although the present data justify no assumption of exact proportionality.

* See this number of the proceedings p. 175.

93 (1271)

Immunization against cyanolophia.

By RHODA ERDMANN.

[*From the Osborn Zoölogical Laboratory of Yale University, New Haven, Conn.*]

When in June, 1915, I was introduced to the study of *Cyanolophia* by A. von Wassermann, Berlin, I performed, under his guidance, several series of experiments to immunize against this chicken disease caused by a filtrable virus. We tried to establish active immunity by treating the animals with attenuated inoculated brain, attenuated inoculated liver, attenuated serum inoculated into bone marrow and virulent sera of different ages and different strengths. The attenuation was effected either by "cultivating" the virulent tissues in chicken plasma after the tissue culture method or by weakening the virulent serum by "cultivation" in bone marrow and chicken plasma. Our tentative experiments, from June, 1915, to October, 1915, did not give results. Only one chicken resisted an inoculation of virulent brain after the following treatment. This chicken, No. 13, had received two inoculations with serum which was obtained 17 hours after the inoculation of virulent brain into another animal. We applied one inoculation with attenuated brain tissue and later bone marrow with attenuated serum was implanted under the skin. After a short interval this animal received another serum treatment and then two days later a lethal dose of virulent brain. It survived this strongest test, while a control chicken which was inoculated with the same brain, but had not undergone these different treatments, died in due time. The immunity of No. 13 was gained without intention. Owing to the scarcity of chickens to be used for experimental purposes at this time in Germany, we had always used the same chickens for our experiments, after the preceding inoculations with the different attenuated materials proved ineffective. So we did not expect No. 13 to live after we inoculated it with virulent brain. We intended it to die for affording virulent serum. As closely as possible we repeated the same treatment, which we had applied to No. 13

by chance, with four other chickens but without success. We could only note a retarding of the incubation period. But with serum No. 13 we could gain passive immunity of an untreated chicken, No. 21.

At this stage of my work I left Germany and continued my experiments in this country at the Osborn Zoölogical Laboratory of Yale University, from October, 1915, up to the present time.

My problem was to find out which of these various factors von Wassermann and I had used in Germany were necessary to produce active immunity, which were not necessary and could be omitted, and which new factors had to be added to produce immunity—not by chance but by a graded series of experiments. It was not necessary to try to gain immunization with serum treatment alone because these experiments had been made by various authors. (See Maggiora and Valenti(1).) Also it was not necessary to try to gain immunization by a treatment with virulent brain alone which had been attenuated by staying long periods in glycerine. (See von Prowazek (2).) Nor did it seem advisable to try to gain immunization by applying to the chickens doses of desiccated brain alone. (See Kraus (3, 4, 5).) My own experience, which I reported here in May, 1916, in trying to immunize with attenuated serum and bone marrow tissue culture alone had not been successful. I could only show that the incubation period could be prolonged. It was certain that our known methods of immunization ought to be varied or combined because none of them alone produced results. Only the knowledge that there is an active immunity induced me to go on in a rather empirical way guided by the idea that it must be possible to raise the resistance of the chicken against *Cyanolophia* by slight, nearly unnoticeable attacks of the disease.

From October, 1915, to July, 1916, I inoculated chickens with desiccated brains on a large scale. These brains[1] had been taken from chickens which died of *Cyanolophia* after a *very prolonged* incubation period. Of these brains I had six which are called in the following Table No. 1, Des. 1 to Des. 6. Table 1 gives an exact survey of the chickens and shows how often each one had been inoculated with the different desiccated brains.

[1] N. B. For details concerning the method of preparing the different protective materials, the exact amount of them, etc., see the main paper.

TABLE I.

Des. 1.	Des. 2.	Des. 3.	Des. 4.	Des. 5.	Des. 6.
11**	19	15****	28	15	15**
13** died	40	19 died	29	28	30**
15	69	24	30	29	31**
16	71	25	32	32	32**
29		28	33	33	33**
30		29**	34	34	40
50		30	40**	54	54**
68		31	46	55	55**
83		32**	54	65**	65**
84		33**	55**	67	67**
85		34**	65	68*	68**
87		40**	67	70***	70
89		41	68	72**	72
		43	70	75	75
		45	72	76	77
		46	75	77	79
		54***	76	78	80**
		55***	77	79	81**
		65**	78	80	82**
		67	79	81	83**
		68	80	82**	86**
		70	81		88**
		72	82		89
		73	84		90
		74	85		91
		80	86		96
		81	87		97
		87	88		98
			89		99
					100

The same chicken, or different ones, had meanwhile been treated with serum, which had been attenuated in bone marrow growing in a plasma medium (Table II).

TABLE II.

CHICKENS TREATED WITH SERUM ATTENUATED IN BONE MARROW.

Ser. B.	Ser. C	Ser. I.	Ser. M.	Ser. N.	Ser. O.	Ser. T.	Ser. V.	Ser. H_1.
1 died	3a	3a	3a**	3a	9	3a		28
2 died	4	4**	4	4	10 died	4		29
3 died	5 died	6 died	7**	7 died		3		30
		7**	8	8			17** died	31
		8					18	32
							19	33
								34
								40
								54
								55
								65
								68

Also during this time, the same or different chickens were

inoculated with brain tissue which had been during shorter or longer periods in a plasma medium. Table III gives the exact

TABLE III.

O.	X.	No. 47.
11	11	11
12** died	22 died	56 died
13	23 died	57
14	24	58
	25	59
	26 died	60
	27 died	61
	28**	62
	29	63
	30	64
	31	65
	32	
	33	
	34	
	35 died	
	36	
	37 died	
	38	
	50	

numbers and shows how often these chickens had been inoculated with virulent brain in a plasma medium.

A few chickens were treated with virulent sera which had been kept on ice (Table IV).

TABLE IV.

Serum O.	Serum T.	Serum Q.	Serum R.	Serum Z.
24	24	24	52 died	67
25	25	25		73
28	28			74

From the chickens prepared in this prescribed manner, I chose from the different groups several animals. For example: No. 15 and No. 77 had received only desiccated brain, No. 15 nine times and No. 77 six times. Both died, together with control animal No. U_1, after being inoculated with virulent brain No. Q. No. 11 and No. 68 which had been treated with desiccated brain and with brain which had been attenuated in a plasma medium, died, together with control animal S_1, after application of virulent brain No. Q. That proves that continued doses of desiccated brain and of brain attenuated in a plasma medium do not protect the chicken against *Cyanolophia*.

The combination of attenuated serum in bone marrow and desiccated brain also did not prove protective. Chickens No. 54 and No. 55 died after inoculation with virulent brain Q. Also a combination of attenuated serum and attenuated brain in a plasma medium proved to be a failure (Chicken No. 24) just as well as the combination of attenuated serum and desiccated brain (Chicken No. 73). But in many cases the incubation periods were prolonged. This caused me to believe that in some of my animals I had raised the resisting power, as Chickens 11, 15, 65, and 67 proved which resisted so many treatments. (Compare Table I and Table II.) Before Chickens 11, 15, and 67 underwent their final test, I applied sera of these more resistent chickens to all animals which were left and some of which had not been treated so frequently with attenuated material. Then I raised the resistance again by the following careful treatment. Chickens 30, 31, 32, 33, 40, 57, 65, 70, 80, 81, 84, 86, 87, 88, 89, 90, 91, 96, 97, 98, 99, and 100 were now treated successively between the 3d and 29th of November, with virulent brain, virulent liver, and virulent serum, which had been allowed to grow with embryonic chicken tissues in a plasma medium. When after these new protective treatments all the chickens were again tested, brain Q being inoculated in all of them, only chickens 57, 84, and 88, died, together with control animal A_2. Brain Q killed a normal untreated chicken, after it had been nine months in glycerine, in three to five days. This is not a very virulent brain. But having attained immunization of 22 chickens against a not too virulent brain, the possibility of immunization against a highly virulent brain was given. But at first I made a mistake and took the highly virulent brain No. 15 which had been only one month in glycerine, and applied serum No. 65 and brain No. 15 to chicken No. 100 and lost it by this too severe treatment. Therefore I used at first for the rest of the chickens the less virulent brain No. 21. I prepared them by an inoculation with serum No. 65 and brain No. 21 (which had been in glycerine nine months and killed a normal chicken in between three and four days) to all of them together with a control animal No. K_2. This control animal died together with 86, 87 and 98. I waited now several weeks for the final test. Then all the chickens underwent a renewed

application of serum No. 65 and brain No. Q, and several days later serum No. 65 and brain No. 15 together with control animal O_2. I lost chickens 96 and 99 and O_2. So I kept 8 immune chickens, because I used four of them for experiments which I cannot record here. Chickens 30, 31, 32, 33, 40, 65, 80 and 81 remained, and passive immunity could be easily produced by using, for example, the serum of either 40 or 65. Both sera applied to untreated chickens together with virulent brain No. 21 or No. 15 protected them fully. One chicken four weeks after its first treatment with serum 65 and brain No. 21 was not killed by a renewed application of brain No. 21. So at least the passive immunity lasted four weeks. I could not follow my experiments to an end because I was compelled by circumstances beyond my control to kill the immune chickens on the 23rd of April, 1917. This causes me to publish my results as they are:

They prove that it is possible to raise the resistance against *Cyanolophia* by slow degrees in applying to the animals the attenuated virus. But, and that seems important, the virus must occur in different physiological stages in the body, as already von Prowazeck believed, and the animal has to be protected against all these possible stages of the agent. The form of the agent in serum, in brain and liver tissue and that form which appears when virulent brain and liver tissue or serum is attenuated with embryonic tissue, must be physiologically different, and it seems only logical to immunize against each of these supposed forms.

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94 (1272)

The action of pancreatic vitamine upon the metabolic activity of paramecium.

By GARY N. CALKINS and WALTER H. EDDY.

[*From the Department of Zoölogy, Columbia University, N. Y. City.*]

The following experiments were undertaken to see if vitamines extracted from sheep's pancreas and adsorbed in fuller's earth by the method employed by Dr. W. H. Eddy have any effect on the metabolic activities of free cells.

The free living cells used in the experiments were the descendants of a single individual *Paramecium aurelia*. These were cultivated on half and half pure spring water and 24-hour-old boiled flour water used fresh each day. On this diet the average division rate varied from 1.5 to 2.0 divisions per day at the time the vitamine tests were started.

The vitamine-bearing fuller's earth was kept in capsules prepared by Dr. Eddy. A small dose of this was obtained by moistening the point of a needle in the *Paramecium* culture water and dipping this in the capsule. The adhering granules were then shaken off in the culture water.

It was necessary at first to determine whether or not the fine granules of the fuller's earth would be ingested by the *Paramecium*. For this purpose sterile spring water was used with the earth. It is well known that similar granules of carmine or indigo will be ingested and a few preliminary tests showed that the granules of fuller's earth are similarly taken in and stored in the gastric vacuoles. To be doubly sure of this a quantity of the fuller's earth was stained with neutral red, washed and then supplied as before to *Paramecium*. The bright pink granules in the resulting gastric vacuoles left no doubt that the *Paramecium* will ingest the vitamine-bearing granules.

The individual *Paramecium* to be studied was isolated in a watch glass containing one drop of Great Bear water, one drop of boiled flour water which has been exposed for twenty-four hours, and the usual dose of vitamine-bearing fuller's earth. It was then stored away in a moist chamber and left for twenty-

four hours when the culture was again examined and the number of individuals counted to get the number of divisions. The control individual was isolated in an identical medium but without the fuller's earth. Five double sets prepared and examined in this way were carried on for a period of 10 weeks.

EXPERIMENTS.

1. In the first series of experiments the organisms were kept in their usual medium of hay infusion and the vitamine-bearing fuller's earth was added to this. Five lines of experimental animals and five of controls were carried on for 5 days giving 27 divisions in the control series and 26 in the vitamine set. The results were therefore entirely negative.

2. In the second series boiled flour was substituted for boiled hay. Five sets of control *Paramecium* and five sets treated with vitamine were carried along for 15 days. The results may be best considered in three groups covering 5 consecutive days each, as follows:

	Controls.		Vitamine-treated.	
	Divisions.	Rate.	Divisions.	Rate.
Group *A*. First 5 days	27	1.08	32	1.28
Group *B*. Second 5 days	12	0.48	22	0.88
Group *C*. Third 5 days	21	0.84	24	0.96
Totals	60	0.80	78	1.08

Here with organisms of different grades of vitality there is a very slight increase in the division rate of the vitamine group, but not enough to prove that the vitamines were effective in stimulating nutrition.

3. In both of the preceding series, individuals were taken at random from a culture dish. While all came from the same original individual it was impossible to tell whether young or old individuals were isolated. In this third series of tests this objection was overcome by using sister cells, one for control, the other for vitamine treatment. In this experiment, as in the others, five lines of control animals and five of vitamine-treated animals were carried on, this time for a period of thirty days,

sister cells being isolated every day. The results may be summarized in six groups including 5 consecutive days each, as follows:

	Controls.		Vitamine-treated.	
	Divisions.	Rate.	Divisions.	Rate.
Group *A*. First 5 days	45	1.80	42	1.68
Group *B*. Second 5 days	28	1.12	26	1.04
Group *C*. Third 5 days	16	0.64	24	0.96
Group *D*. Fourth 5 days	30	1.20	25	1.00
Group *E*. Fifth 5 days	19	0.76	24	0.96
Group *F*. Sixth 5 days	15	0.60	19	0.76
Totals	153	1.02	160	1.06

4. The final experiment was introduced to see if the vitamine treatment will stimulate subsequent metabolic activities. Individuals that had been treated for twenty-four hours were isolated in the usual medium without vitamine. Here again 5 lines with controls were carried on for 15 days. The control animals divided 64 times, the post-vitamine animals 63 times with division rates of 0.85 and 0.84 respectively. The minute differences in all these results fall within the limits of experimental error and show that the vitamines as used have no effect on the nutrition of *Paramecium* cells.

95 (1273)

Further observations on pancreatic vitamine.

By WALTER H. EDDY.

[*From the Department of Pathology, New York Hospital, New York City.*]

In connection with the use of pancreatic vitamine two problems have arisen which must be solved if the treatment is to be more than empirical. One is the establishment of a satisfactory method of dosage determination and the other is the explanation of the method of action of the vitamine upon the organism. In the previous paper is reported an attempt to solve the latter problem. In this paper I wish to present certain collated observations bearing upon the dosage problem.

I. A COMPARSION OF PANCREATIC VITAMINE AND YEAST VITAMINE.

In a recent paper Seidell[1] has outlined the result of his studies of autolyzed yeast filtrate. Making use of similar methods and using an arbitrary standard of 2.7 gm. pancreas to each cubic cm. of water extract as my solution I obtain the following results:

TABLE.

	Autolyzed Yeast Filtrate (Seidell).	Pancreatic Prep. I.	Extract Prep. II.
1. Nitrogen in 100 c.c.	2.0 gm.	0.754 gm.	0.644 gm.
2. 5 gm. Lloyd reagent shaken with 100 c.c. extract cont.	0.09 gm. N.	0.150 gm. N.	0.159 gm. N.
3. 5 gm. Lloyd reagent shaken with filtrate from 2. cont.	0.073 gm. N.	0.088 gm. N.	0.102 gm. N.

From experiments conducted by Williams and Seidell[2] they conclude that vitamine probably contains approximately 50 per cent. nitrogen. Other experiments indicate that practically all the vitamine is removed in the first shaking with the Lloyd reagent. My own experiments confirm their view on the latter point. In regard to the first conclusion I have no data. Accepting their assumptions in both particulars it follows that:

	Yeast Filtrate.	Pancreatic Ext.
100 c.c. of extract contains	0.18 gm. Vitamine	0.30 gm. Vitamine.

Both experiments show that the Lloyd reagent adsorbs nitrogenous substances that are not vitamines. Furthermore it is reasonable to suppose that some of the N of the first shaking is not vitamine N. Hence the vitamine content given above must be taken not as the absolute figure but as the maximum content possible.

Seidell has also found that 1 c.c. of yeast filtrate given every other day protects pigeons from polyneuritis, and that these birds consume 30 gm. of food per day. One cubic cm. of yeast filtrate would therefore contain not more than 0.0018 gm. of vitamine, hence he concludes that 0.0018 gm. vitamine to each 60 gm. of food is a proper vitamine ratio for a pigeon, *i. e.*, one containing 0.003 per cent. vitamine. In experiments with rats and babies my aim has been growth rather than antineuritic tests. In my experi-

[1] Atherton Seidell, "The Vitamine Content of Brewer's Yeast," *Journ. Biol. Chem.*, XXIX, 145.

[2] Williams, R. R., and Seidell, A., *J. Biol. Chem.*, XXVI, 431.

ments no minimum amount has been determined as yet. The data at hand, however, figured on the same basis as follows

Rat No. 4. In 10 days received 38 gm. Lloyd and 116 gm. casein diet, *i. e.*, 2 per cent. vitamine. The gain in weight was 13 gm.

Rat No. 5. In 10 days received 20 gm. Lloyd and 94 gm. casein diet, *i. e.*, 1.2 per cent. vitamine. The gain was 15 gm.

Rat No. 20. In 13 days received 13 gm. Lloyd, 433 gm. farina and 240 gr. condensed milk, *i. e.*, 0.11 per cent vitamine. The gain was 20 gm.

Rat No. 31. In 14 days received 7 gm. of Lloyd, 450 gm. farina and 210 gm. boiled milk, *i. e.*, 0.063 per cent. vitamine. The gain was 15 gm.

Rat No. 39. In 14 days received 3½ gm. Lloyd, 450 gm. farina and 210 gm. boiled milk, *i. e.*, 0.031 per cent. vitamine. The gain was 21 gm.

John G. In 21 days received 42 gm. Lloyd, 630 gm. farina and 1,280 gm. condensed milk, *i. e.*, 0.13 per cent. vitamine. The gain was 588 gm.

10 other babies gained, but in no case on less than 0.13 per cent. vitamine. From these results however it seems hardly a desirable method of figuring vitamine needs. Per cent. of vitamine nitrogen would probably be a better basis.

II. Possibility of a Colorimetric Method of Determining Dosage.

In one of his papers Funk noted that the fraction containing the vitamine gives the blue color reaction with the Folin-Macallum uric acid reagent. This fact suggested another possible method of determining dosage. Experiments to date with pancreatic extract have given the following facts:

1. The water extract gives the color reaction.

2. The extract after treatment with the Lloyd reagent (50 gm. to the liter) and removal of the Lloyd by filtration, still gives the reaction but with a marked reduction in intensity.

3. The Lloyd reagent from the first shaking gives the reaction.

4. The Lloyd reagent shaken with the filtrate from 3 gives the reaction but with marked reduction in intensity.

5. An alkaline and an acetic acid extract of the activated

Lloyd reagent both give the reaction and there is a marked lessening in color intensity when the extract is made of the second shaking of Lloyd reagent.

6. That the color is due in part to the vitamine is shown by the fact that a portion of the extract carried through the complete Funk preparation process gives the color strongly.

7. The color can be obtained with the aid of the centrifuge in a clear form usable against a uric acid standard.

These results show that the substance adsorbed by the Lloyd has the power to give the color reaction and indicates that there is also present some substance other than vitamine which has this power. Granting this fact, however, the results offer a method of standardizing dosage that seems to give promise of practical value.

96 (1274)

Lipoid-free immune serum does not produce passive immunity to transplanted tumors.

By **G. L. ROHDENBURG, M.D.** (by invitation).

[*From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director.*]

Although it has been repeatedly attempted, no one has succeeded in transferring immunity to transplantable tumor in animals by injecting the serum of immune animals. Our experiments on passive immunity, duplicating in part the work of others, have also led to negative results, and are recorded simply to add to the existing data.

Serum was obtained from normal rats, from rats immune to inoculations of the Flexner-Jobling rat carcinoma, and from guinea pigs sensitized by three injections of the Flexner-Jobling tumor in doses of 1 gm. These sera were injected daily in doses of 1 c.c. for nine days previous to an inoculation of 0.003 gm. of the Flexner-Jobling tumor, and a control series of non-injected animals was inoculated at the same time, twenty-five animals being used for each series. Four weeks after inoculation, no immunity was demonstrable in any of the treated groups, which showed from 88 to 92 per cent. takes, as compared with 92 per cent. takes in the control groups.

Serum from rats immune to the Flexner-Jobling carcinoma was extracted with chloroform in order to remove the lipoids which, as has been demonstrated by Jobling and his co-workers, inhibit the proteolytic ferments present in that fluid. The lipoid-free serum was injected subcutaneously into a group of 17 rats in doses of 1 c.c. for seven successive days. Three days after the last injection, these animals and a group of seven controls were inoculated with 0.003 gm. of the Flexner-Jobling rat carcinoma.

Three weeks after inoculation, the animals injected with lipoid-free serum showed 100 per cent. takes as compared with 85 per cent. in the controls. It may be concluded, therefore, that lipoid-free serum, like non-lipoid-free serum, when obtained from immune animals, does not cause passive immunity.

97 (1275)

Relative utilization of free palmitic acid, glyceryl palmitate and ethyl palmitate by dogs.

By J. F. LYMAN (by invitation).

[*From the Dept. Agr. Chem., Ohio State University and Sheffield Laboratory of Physiological Chemistry, Yale Univ.*]

Digestibility of fats depends on several factors which may be grouped as: (1) mechanical, *e. g.*, melting point which determines the rate of gastric discharge[1] and, to a considerable extent, the degree of emulsification; and (2) chemical which determines the character of the products of digestion and the rate of hydrolysis, certain esters of fatty acids, *e. g.*, cetyl palmitate,[2] being attacked very slowly by pancreatic lipase. There is strong evidence for the belief that unchanged esters in a finely emulsified form can not be absorbed by the intestinal mucosa.[3] The facts presented here support this view and agree with the thesis of Terroine[4] that absorption of fats is limited by the rapidity of hydrolysis.

[1] Tangl, F. and Erdelyi, A., *Biochem. Zeitschr.*, 34, 94.

[2] Munk, I., *Archiv. f. (Anat. u.) Physiol.*, 1890, 581.

[3] Bloor, W. R., *J. Biol. Chem.*, 15, 105, 1913.

[4] Terroine, E. F., *J. de physiol.*, 1911, 695.

Two dogs were used for each experiment. The fats employed were added to a basal ration of lean beef, cracker crumbs, and agar. The ethyl palmitate used was neutral; melting point 24°; saponification number 198 (theoretical 197.6). Glycerol palmitate was prepared by heating glycerol and palmitic acid together according to the method of Ellis and Rabinovitz.[1] It was a hard solid with a melting point of about 60°. Feces fatty acids were determined by the method of Gephart and Csonka.[2] Utilization was as follows:

	Dog 1, Per Cent.	Dog 2, Per Cent.	Amount Fed per Day, Grams.
Lard	95.8	95.3	40
Ethyl palmitate	56.8	48.2	40
Glyceryl palmitate	91.7	93.1	40
Palmitic acid	81.2	79.1	40

The ether extract of the feces resulting from feeding ethyl palmitate contained 79.4 per cent. unchanged ester, identified by its melting point, and 20.6 per cent. free palmitic acid. 0.9 per cent. palmitic acid combined as soaps was found.

The poor absorption of ethyl palmitate we think due to a slow hydrolysis as indicated by the large amount of unsplit ester recovered in the feces. Frank[3] has reported a utilization of 75 per cent. for ethyl palmitate; however, the dogs used in his experiments were purged by the large amounts fed and a loss of feces might have resulted in spite of care in collection. The good utilization of glyceryl palmitate is in marked contrast to the poor digestibility of tristearin (9 to 14 per cent.) reported by Arnschinck.[3] It may be worthy of note in this connection that Frank[4] found ethyl stearate utilized to a much less extent than ethyl palmitate, 12 and 75 per cent. respectively. The utilization of glyceryl palmitate is perhaps better than would be expected for such a high melting fat. Mutton tallow, however, having a melting point of 49° is well utilized by dogs (93 per cent.) as found by Arnschink and others.

[1] Ellis and Rabinovitz, *J. Ind. and Eng. Chem.*, 8, 1105, 1916.

[2] Gephart, F. C. and Csonka, F. A., *J. Biol. Chem.*, 19, 521, 1914.

[3] Arnschink, L., *Zeitschr. f. Biol.*, 26, 434, 1890.

[4] Frank, O., *Zeitschr. f. Biol.*, 36, 568, 1898.

98 (1276)

The non-protein nitrogenous constituents of normal human muscle.

By ALMA HILLER, SAMUEL W. CLAUSEN and HERMAN O. MOSENTHAL.

[*From the Johns Hopkins Hospital, Baltimore, Md.*]

The material for these analyses was obtained at operations for severe lacerations, carcinoma of the breast, or gangrene of the extremities. Only such specimens were utilized as were not involved by the pathological process. This seemed to be the only available source from which muscle tissue that might be regarded as normal could be procured. In every case the blood of these subjects was analyzed for its non-protein nitrogen as well as urea content. Any patients in whom these were above the normal would not have been utilized in this series. As a matter of fact, no abnormal bloods were encountered among these cases.

The maximal, minimal and average figures, as well as the number of determinations for each substance, are given in the appended table.

THE NON-PROTEIN NITROGENOUS CONSTITUENTS OF NORMAL HUMAN MUSCLE.

Substance.	No. of Determinations.	Mgms. per 100 Gms. Muscle.			Remarks on Methods.
		Maximal.	Minimal.	Average.	
Non-protein nitrogen..	19	234	100	166	Alcoholic extract.
Non-protein nitrogen..	7	346	265	292	Extraction with heat and acetic acid, evaporation to small volume and extraction with trichloracetic acid.
Urea nitrogen	19	25	8	13	Alcoholic extract.
Kreatin	12	404	212	350	Folin's method.
Kreatinin	13	12	2	5	Folin's method.
Amino acid nitrogen...	12	42	16	32	Van Slyke's method.

The results for the non-protein nitrogen are much lower than they should be. These data were obtained by extracting the muscle tissue by alcohol. Alcohol does not dissolve the kreatin. Since this substance forms such a large portion of the non-protein

nitrogen of muscle, a considerable error is introduced. It is our aim in completing this series of determinations to employ trichloracetic acid or some other fluid for extraction which will approximate the true values more closely.

It is hoped that these studies may form a basis for comparison with pathological muscle tissue.

99 (1277)

The rôle of autolysis in infarction.

By **D. C. STRAUS** and **MAX MORSE.**

[*From the Nelson Morris Memorial Institute for Medical Research, Michael Reese Hospital, Chicago.*]

Are the conditions which are believed to be necessary for autolysis realized in infarction? A true acidity is known to be necessary in some critical cases for autolysis,[1] that is, $P_H < 7, 0$. Infarction was made by kidney vessel ligation. The C_H was determined for the control blood and for that of the blood from the kidney after various periods of time elapsing after ligation.

Time.	PH.[2]
Control from the normal kidney vein	7.2
45 minutes after ligation	6.0
Control minutes after ligation	7.2
After four hours' ligation	6.0

Again, in a guinea-pig liver, excised and frozen by CO_2 within 50 seconds after excision, ground up and suspended in 0.9 per cent. NaCl solution and introduced into a Clark (W. M.) shaking hydrogen electrode C_H gave P_H 6, 5, the blood control giving 7, 2. After 35 minutes, $P_H = 6, 3$.[3] This rapid rise in C_H is in harmony with the observations of Hopkins, Moore and Roaf, concerning the origin of lactic acid immediately after the death of the tissue. It is likewise compatible with the determinations which Taschiro[4] has made on CO_2 evolution after injury. The conclu-

[1] Morse, Max, "Enzyme and reaction of medium in autolysis," *Journ. Biol. Chem.*, 1917, XXX, 197.

[2] By the Sørensen colorimetric method.

[3] By the potentiometer method.

[4] Taschiro, S., "Chemical Sign of Life," Chicago, 1917.

sion is justified that as far as reaction is concerned, the concentration of hydrogen ions is adequate for autolysis.

Can protein hydrolysis be demonstrated coördinate with the C_H? In the experiments described above, the variation of C_{NH_2} nitrogen was found to be as follows:

Time.	Mgm. Per Cent.
Control, vein	7.7
Control, mesenteric vein	7.7
After 45 minutes ligation	8.8
After 240 minutes ligation	12.1

The conclusion is here justified that there is evidence of hydrolysis of the proteins correlative with the development of acidity.

In work with inorganic colloids and catalysis, it has been shown that there is a relation between colloidal dispersion and catalysis. It is known that brain tissue hydrolyzes very much more slowly *in vitro* than *in vivo* and than other tissues. The question arose as to the following point:

Can the autolysis of slowly-autolyzing tissues such as the brain, be accelerated by modifying the dispersion of the colloids? Dog's brain was extracted in the cold with ethyl ether and the residue tested against control tissue from the same brain:

Initial.	7 Days.
Control. 0.55 mgm.	1.25 mgm. NH_2 nitrogen (Van Slyke gasometric method).
Ether... 0.67 mgm.	0.65 mgm. NH_2 nitrogen (Van Slyke gasometric method).

No acceleration (but rather an inhibition) of autolysis occurred. In the use of $CHCl_3$, a similar result was obtained. Again, by saturating the unsaturated compounds of serum, which inhibits autolysis to be some degree, no difference between control and experiment was obtained; the compounds were hydrogenated and iodized by distributing the serum upon the walls of a large separatory funnel.

100 (1278)

Types of anaphylactic reaction.

By W. H. MANWARING and HAROLD E. CROWE.

[*From the Department of Bacteriology and Experimental Pathology, Leland Stanford Jr. University.*]

Study of isolated anaphylactic lungs by perfusion methods[1] shows that there are three types of pulmonary anaphylactic reaction:

(*a*) *Bronchial Anaphylaxis*, or the spasmodic contraction of the bronchial musculature, unassociated with recognizable changes in the pulmonary blood vessels. This type of reaction is illustrated by the lungs of actively sensitized and actively immunized guinea pigs, and by the lungs of guinea pigs passively sensitized with homologous serum.

(*b*) *Vascular Anaphyalxis*, or the spasmodic contraction of the pulmonary blood vessels, usually accompanied with edema. The vascular reation is usually followed by a mild bronchial reaction. This type of reaction is illustrated by the lungs of guinea pigs passively sensitized with heterologous serum, and by the reaction of normal lungs to certain protein split-products and incubated blood mixtures.

(*c*) *Pseudo-Anaphylaxis*, or the plugging of the pulmonary blood vessels with thrombi and agglutinated corpuscle masses.

101 (1279)

Passive cellular anaphylaxis.

By W. H. MANWARING and HAROLD E. CROWE.

[*From the Department of Bacteriology and Experimental Pathology, Leland Stanford Jr. University.*]

Tests of passively sensitized guinea pigs, by perfusion methods, show that the cellular reactions of lungs passively sensitized with homologous serum are apparently identical with those of actively sensitized lungs.

[1] W. H. Manwaring and Yoshio Kusama, *Journ. Immunology*, II, 1917, 157.

It is therefore not necessary to assume the local formation of antibodies in order to account for the active sensitization of pulmonary tissues.

102 (1280)

The rôle of hepatic tissue in anaphylaxis.

By W. H. MANWARING and HAROLD E. CROWE.

[*From the Department of Bacteriology and Experimental Pathology, Leland Stanford Jr. University.*]

If 0.25 per cent. goat serum in 50 per cent. defibrinated normal blood is repeatedly perfused through the liver of a normal guinea pig, a slight reduction in the toxicity of the perfusion fluid is usually observed, on subsequent tests with isolated anaphylactic lungs. In no case, however, is the reduction in toxicity sufficient to prevent the anaphylactic reaction in these lungs.

If the liver of an anaphylactic guinea pig is similarly perfused, the perfusion fluid usually becomes almost completely non-toxic for anaphylactic lungs.

This reduction in toxicity is not accompanied by a measurable decrease in the amount of goat protein in the perfusion fluid, as determined by a subsequent titration with a specific precipitin.

103 (1281)

The food value of soy bean products.

By THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

[*From the Laboratory of the Connecticut Agricultural Experiment Station and the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Connecticut.*]

Soy beans fed as the sole source of protein, or as a supplement to corn gluten, are suitable for the nutrition of rats. They contain sufficient water-soluble vitamine to promote normal growth; for diets containing soy bean flour, butter fat, starch, and an artificial salt mixture have promoted growth as well as comparable rations containing natural protein-free milk. The pres-

ence or absence of the fat-soluble vitamine has not yet been ascertained. The mineral constituents of the soy bean are inadequate for growth. Whether the deficiency is a qualitative or quantitative one remains to be determined. Rats eat foods containing commercial soy bean flour more readily than those containing meal made by grinding the entire seed. The latter is non-toxic; for the few animals which have eaten enough have grown well. Preliminary experiments indicate that the heating to which the commercial soy bean flour is subjected may be the cause of the superiority of the latter. Unlike cotton seed, soy beans extracted with ether are not improved in nutritive value. Unheated soy bean meal and corn gluten has proved satisfactory as the sole source of protein in the diets of chickens. We are continuing our investigation of the nutritive value of this seed.

104 (1282)

The determination of hemoglobin.

By WALTER W. PALMER (by invitation).

[*From the Hospital of the Rockefeller Institute for Medical Research, N. Y.*]

The method for the determination of hemoglobin described below has proved accurate and convenient. 0.1 c.c. of blood is introduced into a 10 c.c. volumetric flask half filled with 0.4 per cent. ammonia water (4 c.c. strong ammonia in 1 liter of water) and filled to the mark with the ammonia solution. The contents are poured into a large test tube (25×200 mm.) and illuminating gas bubbled at a rapid rate through the hemoglobin solution for at least 30 seconds. The resulting carbon monoxide hemoglobin is then compared with a standard solution in a colorimeter (Duboscq).

The standard is a 1 per cent. solution of defibrinated ox or human blood having an oxygen capacity of 18.5 per cent. which has been thoroughly saturated with carbon monoxide. The oxygen capacity may conveniently be determinated by the method described by Van Slyke in the PROCEEDINGS of this Society, 1917, XIV, 84. It has been found convenient to prepare a 10 or 20

per cent. solution of a standardized CO saturated blood kept sealed in the ice chest from which a 1 per cent. standard may be made from time to time. The standard solutions must at all times be protected from bacteria and kept saturated with CO, also controls should be made from time to time with blood of known oxygen capacity. The 0.4 per cent ammonia solution is also used in making up the standard.

RECAPITULATION OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE COMMUNICATIONS.

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1260. [with **M. J. Schlesinger.**] Cholesterinized alcoholic extracts versus acetone insoluble fraction of pure tissue lipoids as antigen for Wassermann reaction.

1261. [with **M. J. Schlesinger.**] The effect of temperature on the rate of complement fixation.

Bullock, F. D.

1250. The influence upon the Ehrlich sarcoma of stimulation or depression of the oxidative processes in mice.

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1191. See **Dragstedt, L. R.**

Burnett, Theodore C.

1218. Note on a toxic nucleoprotein obtained from rat carcinoma.

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1272. [with **Walter H. Eddy.**] The action of pancreatic vitamine on the metabolism of paramecium.

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1223. [with **Richard Weil.**] The excretion of Congo Red by the stomach.

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1276. See **Hiller, Alma.**

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1184. See **Murlin, J. R.**

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1206. See **Whipple, G. H.**

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1184. See **Murlin, J. R.**

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1280. See **Manwaring, W. H.**

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1187. See **Wiggers, C. J.**

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1207. Botulism.

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1246. The non-protein nitrogen of blood: (1) The removal of the protein. (2) The estimation of creatin.

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1181. Cardio-respiratory involvement in infantile scurvy.

1237. A consideration of the reduction of blood platelets in purpura.

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1276. [with **Samuel W. Clausen** and **Herman O. Mosenthal.**] The non-protein nitrogenous constituents of normal human muscle.

Holmes, S. J.

1242. The development of pieces of *Diemyctylus* embryos.

Hoskins, E. R.

1225. [with **Margaret Morris.**] On thyroidectomy in amphibia.

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Janney, N. W.

1239. [with **V. I. Isaacson.**] The influence of thyroidectomy on the blood sugar.

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1195. [with **Josephine B. Neal.**] Quantitative chemical studies in spinal fluids.

Killian, J. A.

1198. [with **V. C. Myers.**] A method for the determination of the diastatic activity of the blood with some observations obtained in diabetes and other conditions.

King, C. E.

1241. A note on the parenteral administration of starch.

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1249. See **Swift, Homer F.**

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1214. [with **M. A. Rothschild.**] Abnormalities in the Q R S group of the electrocardiogram associated with myocardial involvement.

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1281. [with **Lafayette B. Mendel.**] The food value of soy bean products.

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1282. The determination of hemoglobin.

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1259. See **Wallace, George B.**

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1248. The response of the respiratory mechanism to rapid changes in the reaction of the blood.

Pfeiffer, J. A. F.

1179. A note concerning strains of *Treponema pallidum* obtained from the brains of paretics at autopsy.

Pike, F. H.

1201. [with **Helen C. Coombs.**] The postural activity of the rectus abdominis muscle of the cat.

1226. The effect of decerebration upon the quick component of labyrinthine nystagmus.

Prince, A. L.

1258. On the compensation of the ocular and equilibrium disturbances which follow unilateral removal of the otic labyrinth.

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Roper, Joseph C.

1210. See **Eddy, Walter H.**

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1227. See **Stewart, G. N.**

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1264. See **Stewart, G. N.**

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1274. Lipoid-free immune serum does not produce passive immunity to transplanted tumors.

Spencer, Mary W.

1229. See **Klotz, Oskar.**

Stewart, G. N.

1227. [with **J. M. Rogoff.**] The influence of certain conditions on the rate at which epinephrin is liberated from the adrenals into the blood.

1228. [with **J. M. Rogoff.**] The proportion in which adrenalin distributes itself between corpuscles and serum in relation to the technique of testing for epinephrin in the blood.

1263. [with **J. M. Rogoff.**] Relation of the spinal cord to the spontaneous liberation of epinephrin.

1264. [with **J. M. Rogoff.**] Quantitative experiments on the liberation of epinephrin from the adrenals after section of their nerves with special reference to the question whether epinephrin is indispensable for the organism.

Straus, D. C.

1277. [with **Max Morse.**] The role of autolysis in infarction.

Swanson, A. M.

1240. See **Salant, William.**

Swift, Homer F.

1249. [with **Ralph A. Kinsella.**] Active immunization with sensitized and non-sensitized bacteria.

Tower, R. W.

1209. [with **C. F. Herm.**] The intranuclear origin of the mammalian red blood corpuscles as observed in living cultures.

Tsen, Edgar T. H.

1245. On the isolation of streptococci from rabbits.

Uhlenhuth, Edward.

1234. A contribution to the metamorphosis of skin in amphibians.

Umeda, N.

1199. [with **A. I. Ringer.**] Studies in experimental nephritis.

Underhill, Frank P.

1231. [with **Norman L. Blatherwick** and **Samuel Goldschmidt.**] The influence of subcutaneous injections of morphine upon the hydrogen ion concentration of the urine in the dog and rabbit.

EXECUTIVE PROCEEDINGS

Main Society.

Seventy-seventh Meeting.

Cornell University Medical College, October 18, 1916. President Jacques Loeb in the chair.

Members present: Auer, Banta, Benedict, DuBois, Eddy, Eggleston, Gies, Githens, Harris, Hatcher, Hess, Howe, Jackson, Lambert, Loeb, J., Lusk, MacNeal, Murlin, Myers, Ottenberg, Pappenheimer, Ringer, Sherman, Swift, Uhlenhuth, Wiggers.

Members elected: G. A. Baitsell, Henry Gray Barbour, Louis Baumann, W. L. Holman, S. J. Holmes, C. H. Hooper, S. H. Hurwitz, J. W. McMeans, John H. Musser, L. F. Rettger, C. L. A. Schmidt.

Resignation: Edwin O. Jordan.

Seventy-eighth Meeting.

New York Post Graduate Medical School, November 15, 1916. Vice-President Gies in the chair.

Members present: Auer, Bull, Coleman, Eddy, Fine, Gies, Greenwald, Hess, Howe, Jackson, Kleiner, MacNeal, Myers, Oppenheimer, Pike, Ringer, Scott, E. L., Sherman, Wollstein.

Members elected: J. Gardner Hopkins, Miriam Olmstead, J. A. F. Pfeiffer, Arthur L. Meyer.

Seventy-ninth Meeting.

Rockefeller Institute for Medical Research, December 20, 1916. President Jacques Loeb in the chair.

Members present: Auer, Berg, Cohn, Dochez, DuBois, Eddy, Epstein, Fitzpatrick, Flexner, Githens, Jackson, Janney, Kleiner, Kline, Levene, Lewis, Loeb, J., Longcope, Lusk, Meltzer, Meyer, Myers, Noble, Oppenheimer, Ringer, Uhlenhuth, Wallace.

Members elected: C. H. Bailey, William W. Browne, Solomon Strouse.

Eightieth Meeting.

College of Physicians and Surgeons, January 17, 1917. President Jacques Loeb in the chair.

Members present: Auer, Burton-Opitz, Cohn, A. E., Cooke, DuBois, Eddy, Edwards, Field, Gies, Githens, Howe, Jackson, Lee, Lieb, Loeb, J., MacNeal, Myers, Norris, Oppenheimer, Pike, Scott, E. L., Senior, Uhlenhuth, Weil, Williams, H. B.

Members elected: E. R. Hoskins, F. C. McLean, W. L. Niles, J. H. Northrop, W. J. V. Osterhout.

Eighty-first Meeting. (Fourteenth Annual Meeting.)

College of the City of New York, February 21, 1917. President Jacques Loeb in the chair.

Members present: Atkinson, Auer, Berg, Edwards, Funk, Gies, Goldfarb, Greenwald, Hess, Jackson, Janney, Kirkbride, Kleiner, Kober, Lee, Loeb, J., Lusk, MacNeal, Meyer, A. L., Myers, Oppenheimer, Ringer, Scott, G. G., Uhlenhuth, Wallace, Wiggers.

Members elected: George Fahr, M. A. Rothschild.

The meeting was held at 5.00 P. M., and was followed by a dinner at 7.15 P. M. Election of officers occurred for the ensuing year after the dinner and resulted as follows:

President, William J. Gies; Vice-President, John Auer; Secretary-Treasurer, Holmes C. Jackson; additional members of the Council, E. F. DuBois and George B. Wallace.

Eighty-second Meeting.

Presbyterian Hospital, March 21, 1917. President Gies in the chair.

Members present: Auer, Benedict, Cooke, Eddy, Gies, Githens, Greenwald, Hopkins, Jackson, Kleiner, Longcope, Myers, Swift, Thro, Van Slyke, Wallace, Wiggers, Weil, Zinsser.

Eighty-third Meeting.

University and Bellevue Hospital Medical College, April 18, 1917. President Gies in the chair.

Members present: Auer, Benedict, Funk, Gies, Hoskins, E. R.,

Jackson, Kleiner, Loeb, J., MacNeal, Meyer, A. L., Myers, Pike, Ringer, Senior, Uhlenhuth, Wallace.

Members elected: R. L. Kahn, R. A. Kinsella, C. K. Watanabe.

Eighty-fourth Meeting.

Columbia University, May 16, 1917. President Gies in the chair.

Members present: Auer, Burton-Opitz, Cohn, Cole, Eddy, Erdmann, Gies, Githens, Greenwald, Jackson, Janney, Kahn, Kleiner, Lee, Meltzer, Myers, Ringer, Scott, E. L., Sherman.

Members elected: F. E. Chidester, Max Kahn, A. L. Prince, W. H. Welker.

Pacific Coast Branch.

Thirteenth Meeting.

San Francisco, California, October 4, 1916.

Members present: Addis, Cooke, Dickson, Evans, Hewlett, Lucas, Manwaring, Meyer, Ophüls, Whipple.

Fourteenth Meeting.

San Francisco, California, November 8, 1916.

Members present: Addis, Burnett, Cooke, Dickson, Evans, Hewlett, Hooper, Lucas, Meyer, Ophüls, Swain, Wasteneys, Whipple.

Fifteenth Meeting.

San Francisco, California, December 6, 1916.

Members present: Addis, Burnett, Cooke, Crawford, Dickson, Evans, Hewlett, Holmes, Hooper, Schmidt.

Sixteenth Meeting.

San Francisco, California, February 21, 1917.

Members present: Burnett, Dickson, Evans, Hurwitz, Holmes, Hooper, Meyer, K. F., Ophüls, Schmidt, Whipple.

Seventeenth Meeting.

San Francisco, California, April 4, 1917.

Members present: Addis, Burnett, Dickson, Evans, Hooper, Hurwitz, Lucas, Meyer, K. F., Ophüls, Schmidt, Whipple.

REGISTER OF NAMES AND ADDRESSES OF THE MEMBERS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE.

ABBOTT, ALEXANDER C.....University of Pennsylvania.
ABEL, JOHN J.....Johns Hopkins University.
ADAMI, J. GEORGE.....McGill University, Montreal.
ADDIS, THOMAS.....Leland Stanford University, San Francisco.
ADLER, HERMAN M.....Harvard University.
ADLER, ISAAC.....New York Polyclinic Medical School.
ALLEN, A. REGINALD.....University of Pennsylvania.
ALSBERG, CARL.....U. S. Department of Agriculture, Washington, D. C.
AMOSS, HAROLD L.....Rockefeller Institute for Medical Research.
ANDERSON, JOHN F.....New Brunswick, N. J.
ATKINSON, JAMES P.....Department of Health, New York City.
AUER, JOHN.....Rockefeller Institute for Medical Research.
AUSTIN, J. H.....University of Pennsylvania.

BAEHR, GEORGE.....Mt. Sinai Hospital, N. Y. City.
BAILEY, CHARLES H.....Tufts College.
BAILEY, H. C.....Cornell University Medical College.
BAITSELL, G. A.....Yale University.
BANTA, A. M.....Carnegie Institution, Station for Experimental Evolution, Cold Spring Harbor, Long Island, N. Y.
BANZHAF, EDWIN J.....Department of Health, New York City.
BARBER, W. H.....New York University.
BARBOUR, HENRY G.....Yale University.
BARDEEN, CHARLES R.....University of Wisconsin.
BAUMANN, LOUIS.....University of Iowa.
BENEDICT, STANLEY R.....Cornell University Medical College.
BERG, WILLIAM N.....U. S. Department of Agriculture, Washington, D. C.
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BERGEY, DAVID H.....University of Pennsylvania.
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